PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON SEEDS OF *Delonix regia* (Boojer .Hook.) Raf.,

A dissertation submitted to

THE TAMILNADU Dr. M. G. R. MEDICAL UNIVERSITY

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In partial fulfilment of the requirements for the award of degree of

MASTER OF PHARMACY

IN

PHARMACOGNOSY

Submitted by

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CERTIFICATE

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GLOSSARY

OECD	:	Organization for Economic Co-operation and Development
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals
STZ	:	Streptozotocin
SGOT	:	Serum Glutamic Oxaloacetic Transaminase
ALP	:	Alkaline Phosphatase
TP	:	Total Protein
TC	:	Total Cholesterol
TG	:	Triglycerides
HDL	:	High Density Lipoprotein
LDL	:	Low Density Lipoprotein
VLDL	:	Very Low Density Lipoprotein
EtOH	:	Ethanol
EtAc	:	Ethyl acetate
PE	:	Petroleum Ether
DPPH	:	2, 2-DiPhenyl-1-PicrylHydrazyl
H_2O_2	:	Hydrogen peroxide
WHO	:	World Health Organization
TLC	:	Thin Layer Chromatography
UV	:	Ultraviolet
IR	:	Infrared
HPLC	:	High Performance Liquid Chromatography

MS	:	Mass Spectroscopy
GLC	:	Gas Liquid Chromatography
R_f	:	Relative retention time
RSA	:	Radical Scavenging Activity
ADA	:	American Diabetes Association
GOD-POD	:	Glucose oxidase – Peroxidase
GC-MS	:	Gas chromatography-Mass spectroscopy
MAPs	:	Medicinal and Aromatic Plants
NIDDM	:	Non insulin dependent diabetes mellitus
HNF 4α	:	Hepatic nuclear factor 4α
HNF 1α	:	Hepatic nuclear factor 1α
FFA	:	Formalin; Acetic acid; Ethanol
HbA1c	:	Haemoglubin A1c
NaOH	:	Sodium hydroxide
SGPT	:	Serum glutamic pyruvic transaminase

INTRODUCTION

4. INTRODUCTION

People all over the world are now switching to alternative or herbal medicine for the treatment of various ailments. Herbal medicine is gaining popularity not just because of its effectiveness but also by clear marketing of the herbal drugs, describing them as 'natural products' which can not harm to humans. Healthy healing is an incredible resource for every patient. The interest in medicinal plants has increased due to the chronic disease. The Indian medicinal system *viz*. Ayurveda, Siddha and Unani system predominantly use plant based raw materials in most of their preparations and formulations. According to the World Health Organization, it is estimated that 80% of the population in the developing world rely on traditional medicines derived from medicinal plants. The plant based raw materials are safe, preventive, curative and are particularly useful in achieving the goal of "Health to All" in a cost effective manner.

"Whenever the art of medicine is loved

There also is love of humanity"

- **Hippocrates.**

Thus demand of medicinal plant is increasing in both developed and developing countries (Government of India, Planning Commission March, 2000). With the onset of scientific research in herbals it is becoming clearer that medicinal plants have a potential in today's synthetic era. So the ancient knowledge coupled with scientific principles can come into the forefront and provide us powerful remedies to treat different diseases[1]. The WHO in its 29th and 30th assembly gave formal recognition to the traditional medicine. Pharmaceuticals are prohibitively expensive for most of the world's population, half of which lived on less than $2 U. S. per day in 2002[2]. Use of herbal medicine and herbal

treatment of diseases are on the increase even in the developed countries like Australia, Sweden, Switzerland and Canada[3].

HERBAL MEDICINE IN INDIA

India stands first among all the Asian countries in having the knowledge on traditional system related to the use of plant species and diversity of higher plant species, which is the reason for the use of herbs in different forms in alternative systems of medicine. India has about 45,000 plant species. This huge number of medicinal plants species possess important role in health system. Among these, several thousand plants have been claimed to possess medicinal properties. Although herbal medicine are used in various ailments, often these drugs are improperly used and only few of them have got scientifically documented. Hence plant drug need a detailed study in the light of modern medicine in bringing new herbal chemical entities[4-13].

Origin

Plants, animals and human beings have intimate biological relationships for their very basic needs like air, food, fire and fuel, since remote past. The primitive men during the course of their struggle in the forest under the circumstances of object poverty disease and hunger. To liberate themselves from these suffering they should have looked towards the nature (*i.e.*) plants and this inevitably led to trial and error experimentation and discovery of the healing properties of plants by using wild animals. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected the information on herbs and developed well-defined herbal pharmacopeia. In 20th century, the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. About 25% of the prescription drugs dispensed from plant material[14-16].

Importance

The natural to assume that plants were not only eaten for food but were also used as a source of medicine. Although written and pictorial records of herbalism cover no more than the past 5,000 to 6,000 years, archeologically records clearly show that the knowledge and practise of herbal medicine was highly developed long before the earliest written accounts known to us were made. Plants form the major healing powers in almost all ancient civilization like Egyptian, Chinese, India, Roman and Greek[1].

Foods and herbs as medicine

The majority of medicinal plants found in the world grow wild. As natural habitats worldwide are degraded, overexploited and destroyed, many plants species including medicinal plants face threats to their survival. It is becoming increasingly important to take care of the areas in which they grow and to share knowledge about their usefulness. Involving local people from areas where the plants grow is crucial. In the India many people rely on plants and plant – products for food, medicine and shelter. We need to balance health first through proper nutrition with the help of herbs serving as special foods for both body and mind[17].

Wide spread demand for medicinal plants

The pecuniary value of MAPs related to global trade at over 60 billion USD (Govt. of India, 2000). Popularity increased for medicinal plants, both South Asia and Internationally, this trade is expected to grow to 5 trillion by the year 2050 (FRLHT, 1996). Moreover health benefits, MAPs provide crucial livelihood options for millions of rural people in South Asia, particularly women, tribal people and the very poor[17].

Scientific approach to the herbal medicine

The technology involving extraction, purification and characterization of pharmaceuticals from natural sources is a significant contribution to the advancement of natural and physical sciences. The rapid development of phytochemistry and pharmacological testing methods in recent years, new plant drugs is finding their way into medicine as a purified phytochemicals, rather than in the form of traditional galenical preparations. Phytopharmaceuticals or synthetic drugs derived from phytochemicals have to be ultimately incorporated in suitable dosage form which involves the knowledge of dispensing and preparative pharmacy, pharmaceutical technology and analysis.

Pharmacognosy is a vital link between Ayurvedic and Allopathic systems of medicines. It provides a system wherein the active principles of crude drugs derived from natural origin can could be dispensed, formulated and manufactured in dosage forms acceptable to the Allopathic system of medicine[18].

DIABETES MELLITUS

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycaemia associated with disturbances of carbohydrate, fat and protein metabolism due to absolute or relative deficiency in insulin secretion and/ or action. Diabetes causes long term damage, dysfunction and failure of various organs; especially the eyes, kidneys, nerves, heart and blood vessels.

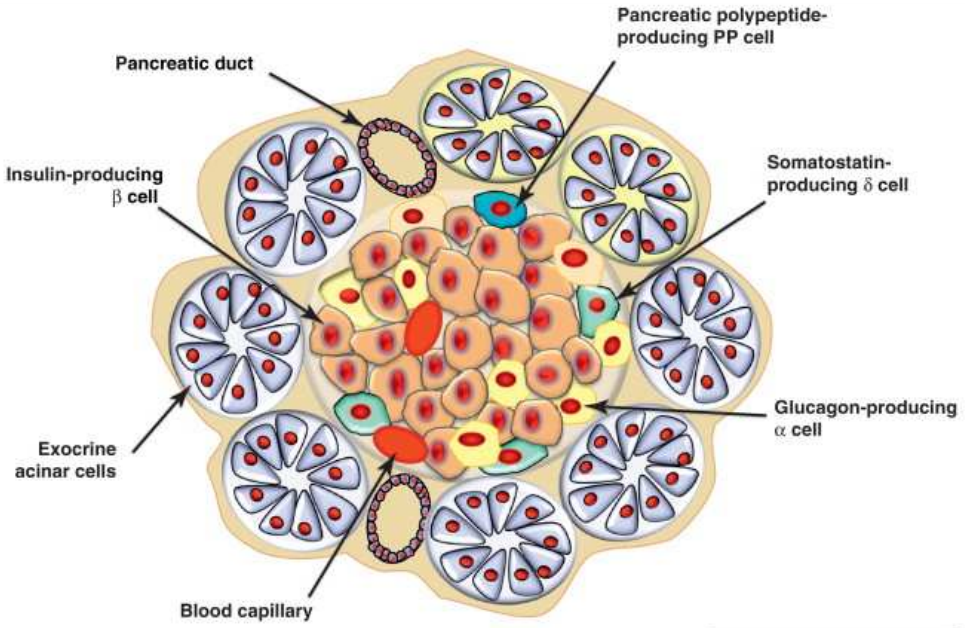

Fig. 1 Pancreas

Diabetes is not a single disease; rather it is a heterogeneous group of syndromes characterized by an elevation of blood glucose caused by relative or absolute deficiency of insulin[19].

Epidemiology of diabetes

The incidence of diabetic is growing rapidly in United States and worldwide. Globally as of 2010, an estimated 285 million people had diabetes, with type II making up about 90% of the cases[20]. In 2013, according to International Diabetes Federation an estimated 381 million people had diabetes[21-22], its prevalence is increasing rapidly. It is estimated that more than 250 million people worldwide are afflicted with diabetes and the prevalence is expected to exceed 350 million by the year 2030[1].

Symptoms of diabetes[1]

Common symptoms include the following:

- Frequent urination
- Excessive thirst
- Unexplained weight loss
- Sudden vision changes
- Head ache, fatigue, lethargy
- Muscle cramps, irritability, emotional liability
- Abdominal discomfort

Types of diabetes[20]

The ADA recognizes four clinical classification of diabetes

- **Type - I diabetes** (insulin dependent diabetes mellitus)
- **Type - II diabetes** (formerly, non-insulin dependent diabetes mellitus)
- **Gestational diabetes** (first recognition during pregnancy)
- **Diabetes due to other causes** (genetic defects or medication)

Type I diabetes

Type 1 diabetes is called as insulin-dependent diabetes. It is also represented as juvenile-onset diabetes, because it often begins in childhood. Type 1 diabetes most commonly afflicts individuals in puberty or early adulthood. The disease is characterized by an absolute deficiency of insulin caused by massive β-cell necrosis. In all type1 cases circulating insulin levels are low or very low and patients are more prone to ketosis. This type is less common and has a low degree of disposition[19-20].

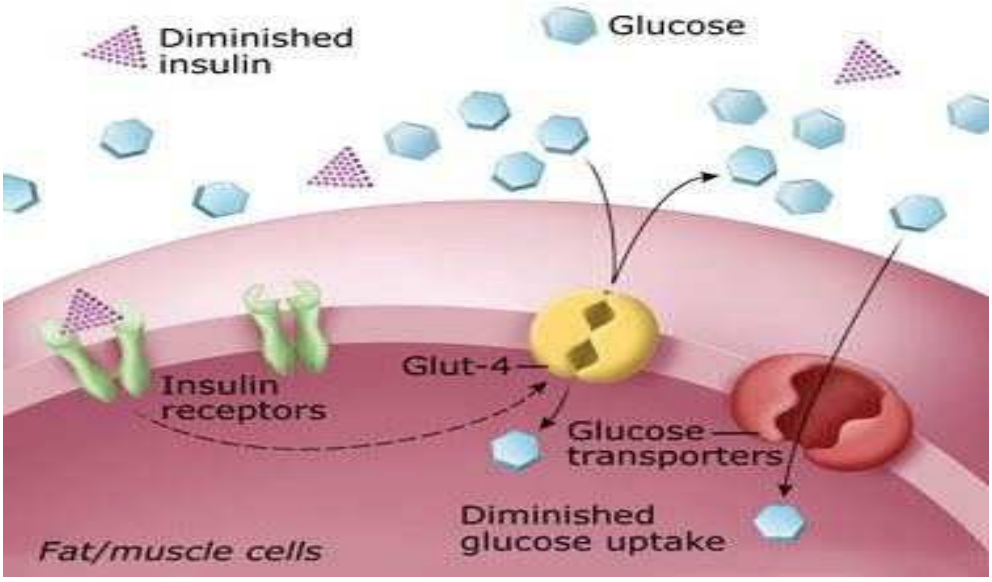

Fig. 2 Type 1 diabetes

Type II diabetes

Type 2 diabetes (NIDDM) is influenced by genetic factors, aging, obesity and peripheral insulin resistance, rather than by autoimmune processes or viruses. Over 90% cases are type 2 diabetes mellitus. Type 2 diabetes is frequently accompanied by the lack of target organs to either endogenous or exogenous insulin. This insulin is considered as major cause of this type 2 diabetes[19-20].

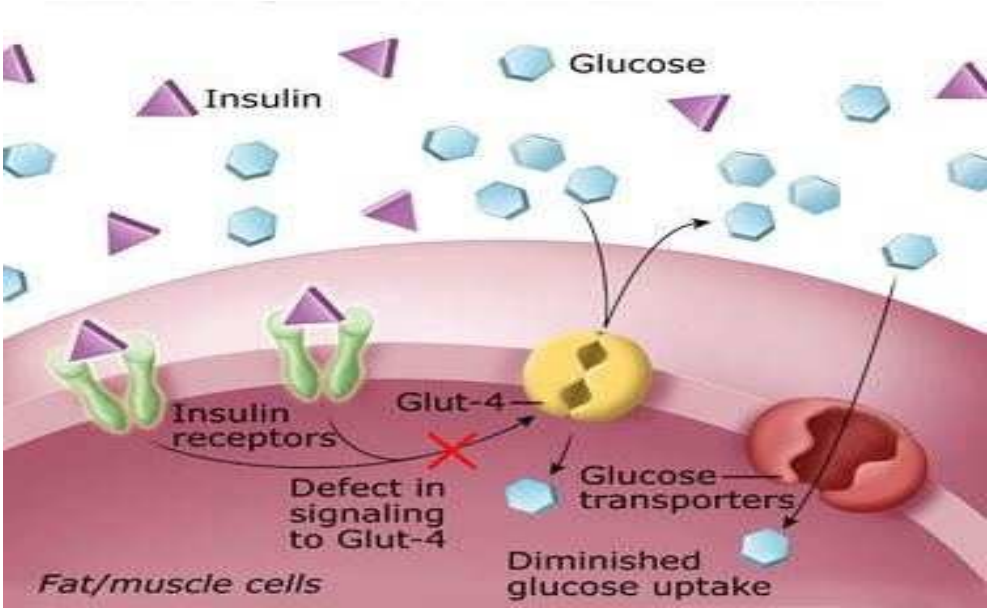

Fig. 3 Type II diabetes

Gestational diabetes

Diabetes that's triggered by pregnancy is called gestational diabetes (pregnancy, to some degree, leads to insulin resistance). It is often diagnosed in middle or late pregnancy. Because high blood sugar levels in a mother are circulated through the placenta to the baby, gestational diabetes must be controlled to protect the baby's growth and development. The rate of gestational diabetes is between 2% to 10% of pregnancies. Gestational diabetes usually resolves itself after pregnancy. Up to 10% of women with gestational diabetes develop type 2 diabetes[19-20].

PATHOPHYSIOLOGY

Pancreas

The hormones play an important role in regulating the metabolic activities of the body, particularly the homeostasis of blood glucose. The pancreas is both an endocrine and exocrine gland, in which endocrine produces the peptide hormone insulin, glucagon and

somatostatin and exocrine gland produces digestive enzymes. The peptide hormones are secreted from cells located in the islet of langerhans (β cells produce insulin, alpha cells produces glucagon and δ cells produce somatostatin)[19-20].

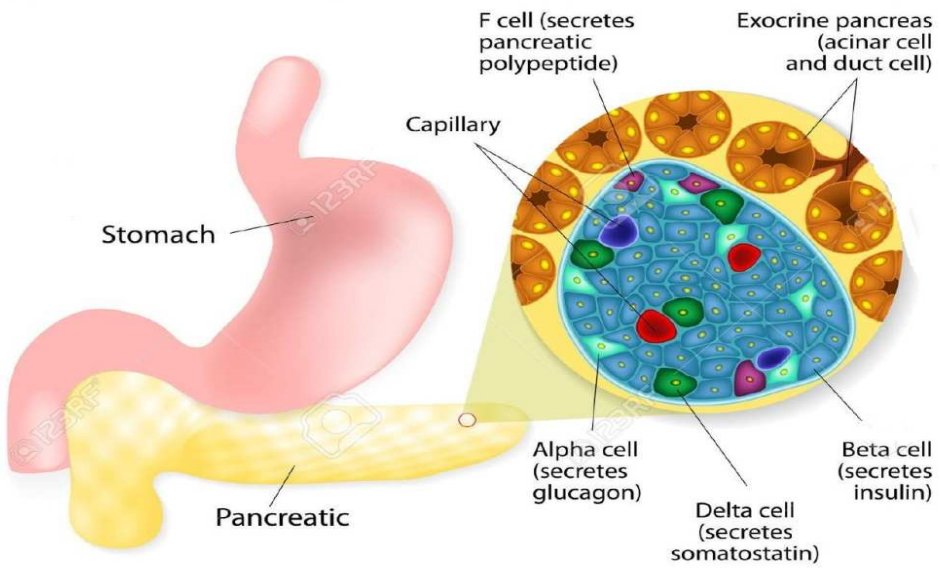

Fig. 4 Langerhans

Genes of diabetes mellitus[1]

Two genes called "MODY 1" and "MODY III" that appear to contribute to the 2% to 5% of diabetes, that are clearly inheritable.

MODY I GENE

Mody I gene is located on chromosome 20, makes hepatocyte nuclear factor -4α (HNF, 4α) a cell receptor that plays a role in HNF - 4α production.

MODY II GENE

Mody II gene is located on chromosome 12 produces hepatocyte nuclear factor-1α (HNF, 1α) a protein found in the liver and β cells of the process.

Insulin[19-20]

Insulin was discovered in 1921 by Banting and best who demonstrated the hypoglycaemic action of an extract of pancreas. In 1922 an extract containing insulin was first used on a 14 year old boy suffering from severe diabetes mellitus with excellent response. Insulin was then purified in a few years.

Chemistry, synthesis and secretion

The islets of langerhans are composed of 4 types of β cells secrete insulin, alpha cells glucagon, δ cells somatostatin and P cells secrete pancreatic polypeptide. Glucose enters the pancreatic β cells with the help of glucose transporters.

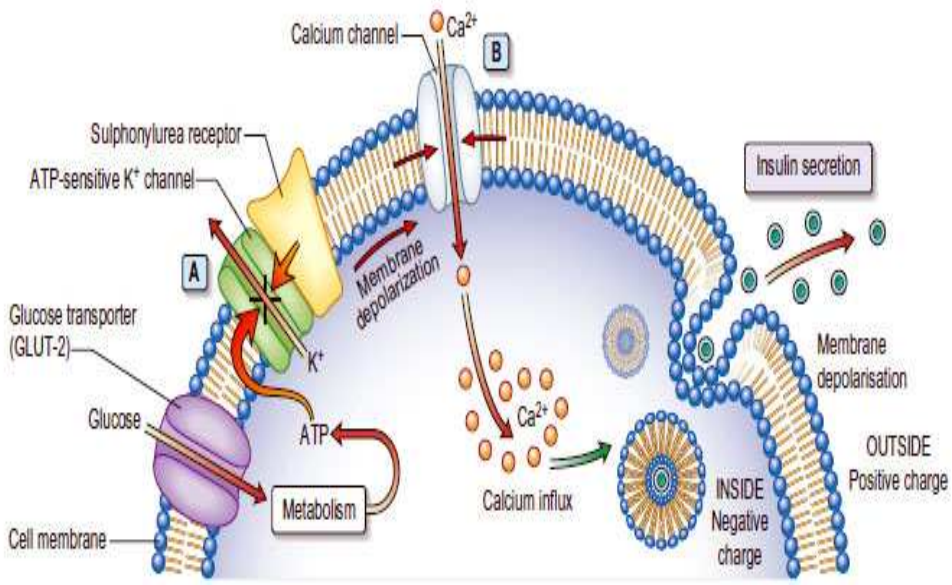

Fig. 5 Secretion of insulin in β cells

Insulin is released from the granules by a process of exocytosis. Natural insulin is a polypeptide synthesized from the precursor preproinsulin which is cleaved to get proinsulin. Proinsulin is processed in the secretary granules to get insulin. It has two

peptide chains-A chain (21 amino acid) and B chain (30 amino acid) linked by disulphide bridges.

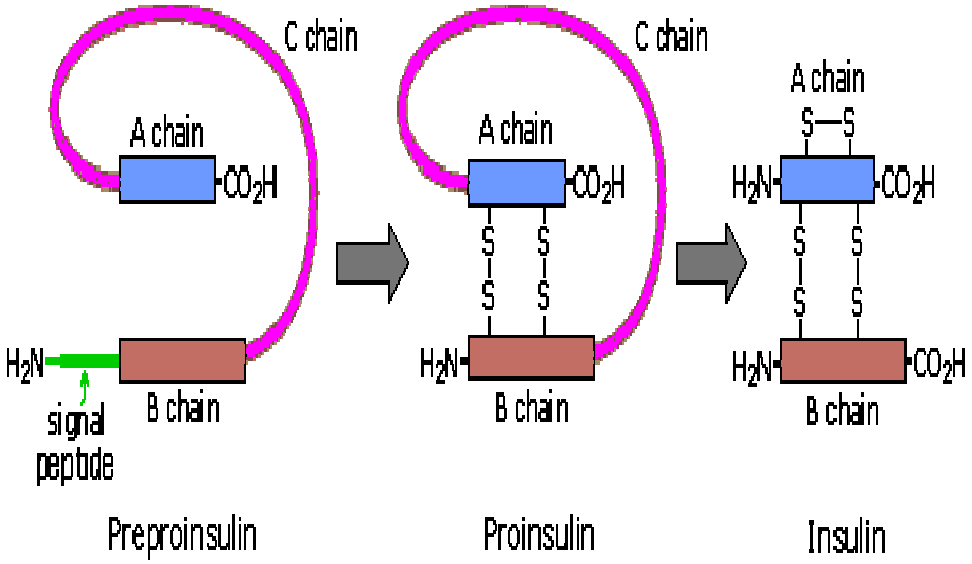

Fig. 6 Structure of insulin

Insulin is stored in granules in the β islets cells of the pancreas. Normal pancreas releases about 40 to 50 units of insulin every day. Basal insulin secretion is 0.5-1U/hr and increase after meals up to 6U/hr. Insulin is metabolised in the liver, kidney and muscle.

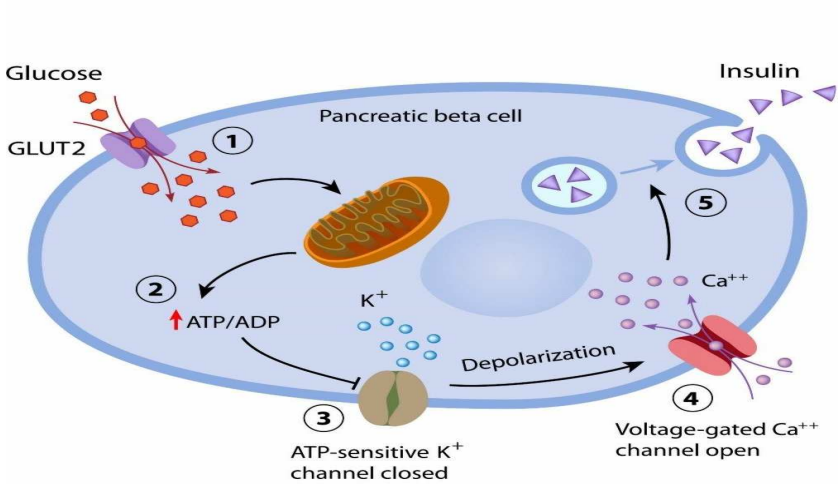

Fig. 7 Insulin release from β islets cells of the pancreas

Mechanism of action

Insulin acts by specific receptors. Insulin receptor is a glycoprotein made up of two α and β subunits. Insulin binds to the alpha subunit of the receptors present on the surface of target cells. This binding stimulates tyrosine kinase activity in the β subunit. This in turn activates a cascade of phosphorylation and dephosphorylation reactions which stimulate or inhibit the enzymes involved in the metabolic actions of insulin.

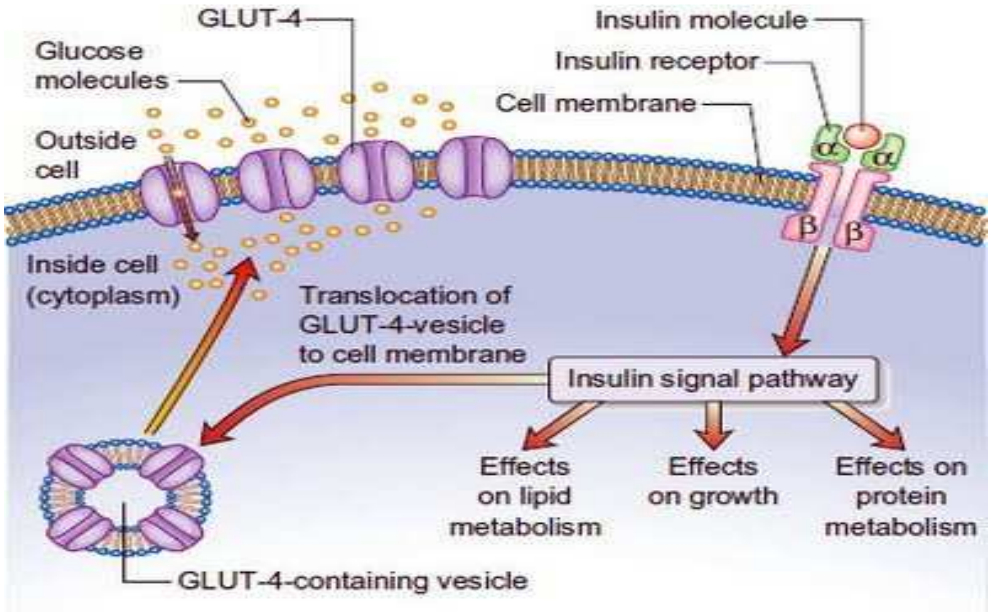

Fig. 8 Mechanism of action of insulin

The following table provides information about the characteristic of different types of insulin and characteristic[23]

Table 1. Types of insulin and characteristics

Insulin	Onset (hr)	Peak (hr)	Duration (hr)
A. Rapid acting			
Lispro	0.25-0.5	0.5-2.5	3-6.5
Asparte	10 -20 min	1-3	3-5
Glusiline	5-15 min	30- 45 min	2- 4
B. Short acting			
Regular	0.5-1	1- 5	6- 10
C. Intermediate acting			
NPH (human)	1-2	6-14	18 - > 24
Lente (human)	2-4	4-12	12-18
D. Long acting			
Ultralenete (human)	6-10	10-6	18-24
Glargine	3-4	-	20-24

Actions of insulin[19]

Insulin target on various organs such as liver, muscles and adipose tissue have been showed on Table. 2

Table 2. Insulin act on various organs

Liver	Muscle	Adipose tissue
Increased glucose uptake and glycogen synthesis	Increased glucose uptake and utilization	Increased glucose uptake and storage as fat and glycogen
Inhibits glycogenolysis and glucose output. Inhibits gluconeogenesis from protein, pyruvate, FFA and glycerol	Inhibits proteolysis and release of amino acids, pyruvate, lactate into blood which forms substrate for gluconeogenesis in liver	Inhibits lipolysis and release of FFA + glycerol which forms substrate for gluconeogenesis in liver

TREATMENT OF DIABETES MELLITUS

The goal of diabetes management is to keep blood glucose levels as close to normal as safely possible. Since diabetes may greatly increase risk for heart disease and peripheral artery disease, measures to control blood pressure and cholesterol levels are an essential part of diabetes treatment as well[24].

Type I diabetes treatment

A person with type 1 diabetes must rely on exogenous (injected) insulin to control hyperglycemia, avoid ketoacidosis and maintain acceptable levels of glycosylated haemoglobin (HbA1c). The use of home blood glucose monitors facilitates frequent self-monitoring and treatment with insulin injections. Continuous subcutaneous insulin infusion is another method of insulin delivery (insulin pump). The pump is programmed to deliver a basal rate of insulin secretion and it also allows the patient to control delivery of a bolus of insulin to compensate for high blood glucose.

Other methods of insulin delivery such as transdermal, buccal and intranasal are currently investigation. Amylin is a hormone that is co secreted with insulin from pancreatic β cells following food intake. Pramlintide a synthetic analogue of amylin may be used as an adjunct to insulin therapy.

Type II diabetes treatment

Treating type 2 diabetes is maintaining blood glucose concentrations within normal limits and to prevent the development of long-term complications of the disease. Weight reduction, exercise and dietary modification decrease insulin resistance and correct the hyperglycemia of type 2 diabetes in some patients. As the disease progresses, β cell function declines and insulin therapy is often required to achieve satisfactory serum glucose levels.

Gestational diabetes treatment

- Treatment during pregnancy includes working closely with your health care team
- Careful meal planning to ensure adequate pregnancy nutrients without excess fat and calories

- Daily exercise
- Controlling pregnancy weight gain
- Taking diabetes insulin to control blood sugar levels if needed.

MANAGEMENT OF DIABETES MELLITUS[1,19-20]

Glycemic Control

There are two primary techniques available to assess the quality of a patient's glycemic control

- Self - monitoring of blood glucose (SMBG) and
- Interval measurement of haemoglobin A1C (HbA1c).

Self-Monitoring of Blood Glucose

SMBG is an effective way to evaluate short-term glycaemia control. It helps to monitor the effect of food, medications, stress, and activity on blood glucose levels of patients. The frequency of checking helps to prevent the risk factor and to maintain the medical therapy. For patients with type 1 and type 2 diabetes mellitus, self monitors their glucose at least three times per day. Initially some patients require more frequent monitoring, including both pre-prandial and postprandial readings.

Patients with gestational diabetes who are taking insulin should monitor their blood glucose three or more times daily. But also the inclusion of health-improving behaviour such as diet and exercise maintained. For pregnant women, glucose levels of 60 to 99 mg/dL and peak postprandial levels between 100 and 129 mg/dL.

Haemoglobin A1c

HbA1c measures no reversible glycosylation of the haemoglobin molecule, which is directly related to blood glucose concentrations. Periodic testing is recommended in all patients with diabetes. The ADA recommends that patients with stable glycaemia control be tested at least twice a year. The frequency of testing depends on the clinical situation and the patient's treatment regimen.

Other management methods are,

- Goals of therapy
- Patient and family education
- Nutrition
- Fibre
- Alcohol
- Artificial sweeteners
- Exercise

Synthetic drug treatment[25-27]

When considering appropriate pharmacologic therapy, a major factor to consider is whether the patient is insulin deficient, insulin resistant, or both. Treatment options can be divided into insulin sensitizers, secretagogues, α glucosidase inhibitors, incretins, pramlintide, SGLT-2 inhibitors, insulin and insulin analogs.

Table 3. Pharmacological treatment

Subgroup	Generic Name(Brand)	Class	Route	Comments
Biguanides	Metformin (Glucophage)	Sensitizer	Oral	Weight loss No hypoglycemia
Thiazolidinediones	Rosiglitazone, (Avandia) Pioglitazone, (Actos)	Sensitizer	Oral	Weight gain Peripheral edema
Alpha glucosidase Inhibitors	Acarbose (Precose), Miglitol (Glyset)	Sensitizer	Oral	GI upset No Hypoglycaemia
Sulfonylureas	Glimepiride(Amaryl) (Orinase), Glipizide (Glucotrol), Tolazamide (Tolinase), Tolbutamide	Sensitizer	Oral	Hypoglycemia Weight gain
Glinides	Nateglinide (Starlix), Repaglinide (Prandin)	Sensitizer	Oral	Weight gain
Exenatide	Byetta	GLP-1 analog	Subcutaneous	Weight loss GI upset
Liraglutide	Victoza	GLP-1 analog	Subcutaneous	Weight loss Nausea
Extended release exenatide	Bydureon	GLP-1 analog	Subcutaneous	Weight loss Nausea
Pramlintide	Symlin	Incretin	Subcutaneous	GI upset Adjunctive therapy with insulin
Dipeptidyl peptidase-4 inhibitors (DPP-4s)	Sitagliptin (Januvia), Saxagliptin (Onglyza), Linagliptin (Trajenta)	DPP-4 Inhibitors	Oral	No hypoglycemia Nasopharyngitis Weight neutral
SGLT-2 inhibitors	Canagliflozin (Invokana) Dapagliflozin	Renal Glycosuria	Oral	Oral Polyuria UTIs

ALTERNATIVE MEDICINE FOR DIABETES[1]

The early developing phase of modern medicine relied on herbs as a major source of drugs. With years of extensive research and isolation of the active agents, herbs had given way to the 'pills of modern medicine'. With immense flora present on the earth, one cannot keep a track of all the herbs and so there can be many herbs and plants whose products may be beneficial in treating disease. Such herbs or plant products should be analysed for their active ingredients which can be used for the manufacture of future drugs.

Table 4. Antidiabetic plants and their curative properties

S. No	Botanical name	Common name	Family	Uses
1.	*Abelmoschus esculentus*	Bhindi	Malvaceae	2 fresh seedless tender fruits are given twice daily till cure
2.	*Buchanania lanzan*	Piyar	Anacardiaceae	Power of leaf or seed helps in controlling glucose levels at early stage of diabetes
3.	*Cyperus triceps*	Nirbishi	Cyperaceae	10 ml of root decoction is given during excessive thirst caused due to diabetes
4.	*Daucus carota*	Gajar	Apiaceae	1 teaspoonful of root powder is given twice a day with hot water for 20 days
5.	*Enicostemma axillare*	Nagaguha	Genetianaceae	Whole plant is used till cure
6.	*Feronia limonia*	Wood apple	Rutaceae	Leaves powder is useful if it is taken on empty stomach every day for 30 days
7.	*Gossypium herbaceum*	Kapas	Malvaceae	5 ml of decoction of the seeds is taken orally 2 times a day for 40 days
8.	*Hordeum vulgare*	Barley	Poaceae	1 or 2 chapati's are taken daily till cure
9.	*Indigofera arrecta*	Bengal indigo	Papilionaceae	Root powder is taken 3 times a daily till cure
10.	*Ipomaea maxima*	-	Convolvulaceae	10 g of roots is made into a paste and given with sugar candy twice daily for 2 – 3 days

As practitioners of evidence based modern medicine, most physicians believe that herbal products could be effectively used for treating disease only after these products are thoroughly tested for the benefits and validated for medicinal use by the concerned agencies. For all these reasons herbs should be taken with care under the supervision of practitioner knowledge in the field of botanical medicine. Folkloric uses are supported by a long history of human experience. Numerous biologically active plants are discovered by evaluation of ethno pharmacological data and these plants may offer the local population immediately accessible therapeutic products.

Although more than 1000 plants have been claimed to offer special benefits in the treatment of diabetes, few have received details scientific investigation, leaving scope for extensive further work.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Pharmacognostical review

1. Sarojini sarangapani *et al .,* (2012) carried out the Pharmacognostical study on *Delonix regia* seed polymer.[28]
2. Barhate. A. N *et al .,* (2014) carried out the Pharmacognostical study on *Delonix regia* seed gum.[29]

Phytochemical review

3. Kavitha Sama *et al .,* (2011) the root bark of *Delonix regia* possess tannin, terpenoid, sterol, glycoside, alkaloid, phenol.[30]
4. Samar. S *et al .,* (2012) carried out phytochemical analysis showed on *Delonix regia* leaves and reported the isolation of seven flavonoidal glycosides.[31]
5. Ravi Kumar. A *et al .,* (2013) the investigation of the *Delonix regia* leaves contains carbohydrate, alkaloid, flavonoid, cardiac glycoside, anthroquinone glycoside, saponins, steroid and tannin.[32]
6. Fatmawaty *et al.,* (2013) bark, leaf and peel extract have alkaloid compounds. Flavonoids found in flowers, phenolic compounds found in Bark and flower of *Delonix regia*.[33]
7. Anindita Biswas *et al .,* (2014) the phytochemical analysis on *Delonix regia* flower bearer alkaloid, flavonoid, sterol, saponin, protein, amino acid, carbohydrate, glycoside, carotenoid, tannin, and phenolic compounds.[34]

8. Rajabhau S Shiramane *et al* ., (2011) reported the phytochemical investigation is showed the *Delonix regia* flowers possess carbohydrate, flavonoid, saponins glycoside, tannin and steroid.[35]
9. Nilam Yadao *et al* ., (2015) reported the phytochemical analysis showed the *Delonix regia* leaves possess carbohydrate, flavonoid, saponins and tannin.[36]
10. Mariajancyrani J *et al* ., (2013) reported the phytochemical analysis showed the *Delonix regia* leaves possess flavonoid, sterol and glycoside.[37]
11. Mohd Asif Khan *et al* ., (2012) reported the phytochemical analysis showed the *Delonix regia* flowers possess carbohydrate, flavonoid, saponins, tannin and steroid.[38]
12. Ghulam Shabir *et al* ., (2011) reported the quantitative analysis showed the *Delonix regia* leaves, flowers and bark possess phenolic acids.[39]
13. Shanmukha I *et al* ., (2011) reported the phytochemical analysis showed the *Delonix regia* flowers possess protein, alkaloid, flavonoid, tannin and phenolic compound. Also reported the total phenolic content.[40]
14. Consolaction Y. Ragasa etal (2011) reported the *Delonix regia* leaves chemical structure was deducted.[41]
15. Aly M. El – sayed *et al* ., (2011) reported the *Delonix regia* flowers chemical structure was established by spectral analysis.[42]
16. Joythi M. Veigas *et al* ., (2007) reported the *Delonix regia* floral petals possess polyphenols compounds was estimated by total quantification phytochemicals.[43]
17. Sarojini sarangapani *et al* ., (2012) reported the phytochemical analysis showed the *Delonix regia* seed polymer possess carbohydrate, alkaloid, steroid, flavonoid, terpenoid, glycoside, sapponin, tannin and fixed oil.[44]

18. Barhate. A. N *et al* ., (2014) reported the physicochemical characterization of *Delonix regia* seed gum for pharmaceutical suspension.[45]
19. Felix Adje *et al* ., (2008) reported the *Delonix regia* flowers bio molecular composition, for pilot – plant scale membrane technology.[46]

Pharmacological review

20. Rajabhau S Shiramane *et al* ., (2011) reported the *Invivo* Antidiarrhoeal activity on *Delonix regia* flowers.[47]
21. Fatmawaty *et al* ., (2013) carried out Antimalarial activity on *Delonix regia* leaves, bark, seeds and flowers.[48]
22. Rozina Paul *et al* ., (2014) carried out Antinociceptive and cytotoxic activity on *Delonix regia* leaves.[49]
23. Nilam Yadao *et al* ., (2015) carried out Antidiabetic and antioxidant activity on *Delonix regia* leaves.[50]
24. Kavitha Sama *et al* ., (2012) carried out Antibacterial activity on *Delonix regia* root bark.[51]
25. Sachidanandha Swamy H. C *et al* ., (2014) carried out Antibacterial activity on *Delonix regia* flowers. [52]
26. Dinanath D Patil *et al* ., (2014) carried out Antibacterial and anti – inflammatory activity on *Delonix regia* leaves and roots.[53]
27. Shiramane Rajabhu S *et al* ., (2011) carried out Gastroprotective activity on Deloninx regia flowers.[54]
28. Anindita Biswas *et al* ., (2014) carried out Hepatoprotective activity on *Delonix regia* flowers.[55]

29. Ahmed Salman *et al .,* (2012) carried out Antiemetic activity on *Delonix regia* leaves.[56]
30. Vivek M.` N *et al .,* (2013) carried out Antimicrobial activity on *Delonix regia* leaves and flowers.[57]
31. Mohd Asif Kan *et al .,* (2012) carried out Wound healing activity on *Delonix regia* flowers.[58]
32. Tagalpallewar. V. R. *et al .,* (2014) carried out Antidiabetic activity on *Delonix regia* leaves.[59]
33. Ghulam Shabir *et al .,* (2011) carried out Antimicrobial activity on *Delonix regia* leaves, flowers and bark.[60]
34. Mohamed Z. M. Salem *et al .,* (2014) carried out Antibacterial and anti fungal activity on *Delonix regia* stem wood and bark.[61]
35. Ode O. J *et al .,* (2013) carried out Antidiarrhoeal activity on *Delonix regia* root bark.[62]
36. Consolacion Y. Ragasa *et al .,* (2011) carried out Antimicrobial activity on *Delonix regia* leaves.[63]
37. Samaresh Pal Roy *et al .,* (2013) carried out Antiulcer activity on *Delonix regia* flowers.[64]

ETHNOBOTANICAL SURVEY

3. ETHNOBOTANICAL SURVEY[65-68]

PLANT PROFILE

Plant name	:	*Delonix regia* (Boojer .Hook.) Raf.
Common name	:	*Royal Poinciana.*
Synonym	:	*Poiniana regia.*
Family	:	Leguminosae.

Vernacular names

English	:	Flamboyant flame tree, Flame of the forest, Gulmohar
Arabic	:	Goldmore
French	:	Flamboyant, Poinciana,royal
Burmese	:	Seinban
Spanish	:	Acacia roja
Swahili	:	Mjohoro, mkakaya
Hindi	:	Gulmohr
Bengali	:	Chura, radha
Tamil	:	Mayarum, mayirkonrai, Perugondrai

Taxonomical status

Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliophyta
Order	:	Fabales

Family	:	Leguminosae
Subfamily	:	Fabales
Genus	:	*Delonix*
Species	:	*Delonix regia*

Origin

Madagascar and Zambia.

Exotic

Brazil, Egypt, India, Kenya Niger, Cyprus, Sri Lanka, South Africa, Uganda, United States of America and Jamaica.

Habitat

The ornamental plant, found in road side and forest.

Habit

Delonix regia (Boojer .Hook.) Raf. Tree is 40 feet in height having umbrella like canopy can be wider than its height.

BIOGRAPHY OF THE PLANT

Fig .9 *Delonix regia* (Boojer .Hook.) Raf. Tree

PLANT DESCRIPTION

Delonix regia (Boojer. Hook.) Raf., is native to Madagascar, where it is almost extinct. This plant is now widespread in most tropical and subtropical areas. Plant can grow at higher altitudes than recommended, but flowering become irregular[69].

Seeds[70, 28]

Seeds are speckled, rather like a fava bean. Typical of most hard seeded legumes, hard, greyish, glossy, to 2 cm long, oblong and shaped very much like date seeds

Fig .10 *Delonix regia* seeds

Bark[65]

Smooth, greyish-brown, slightly cracked and having many lenticels; inner bark is lightbrown.

Fig .11 *Delonix regia* bark

Leaves [64,71- 72]

The compound leaves have a feathery appearance and are a characteristic light, alternate, bright green. Leaflets - Each bearing 12-40 pairs of small oblong-obtuse leaflets that are about 0.5-2 cm long and 0.3 cm wide; petiole stout.

Fig. 12 *Delonix regia* leaves

Flowers[73-74]

Flowers 5-13 cm across, with 5 equal petals, on slender stalks 5-7.6 cm long.

Fig.13 *Delonix regia* flowers

Fruits[67]

Fruit green and flaccid when young, turning to dark brown, hard, woody pods, 30-75 cm long, 3.8 cm thick, 5-7.6 cm broad, with many horizontally partitioned seed chambers inside, indehiscent, finally splitting into 2 parts.

Fig. 14 *Delonix regia* fruits

Ethanobotanical use[44]

Table 5. Ethanobotanical use

***Delonix regia* (Boojer .Hook.) Raf.**	**Ethanobotanical use**
Leaves	Antidiabetic, anti-inflammatory, antimicrobial activity
Flowers	Antidiarrhoeal, anthelminitic, wound healing, Gastroprotective activity
Leaves, flowers and barks	Antioxidant activity
Seeds	Antidiabetic activity
Aerial parts	Hepatoprotective activity

RATIONALE FOR SELECTION OF THE PLANT

4. RATIONALE FOR SELECTION

Diabetes mellitus is a debilitating and often life threatening disease with increasing incidence throughout the world. Diabetes complications arise partly from glycosylation damage to structural and functional protein and reflect chronic failure to maintain blood glucose homeostasis.

In addition to hyperglycaemia, several other factors such as dyslipidemia or hyperlipidemia are involved in the development of cardiovascular complications of diabetes by which 30-40% of diabetic population eventually develop end stage renal failure. Also increased oxidative stress is a widely accepted factor in the progress of diabetes and its complications. Almost 80% of deaths occur in low and middle-income countries. Synthetic drugs used for diabetes have produced adverse effects using longer time when compared to herbal drugs.

Nowadays plants are being upgraded by validating the traditional claims and establishing its medicinal value. Various parts of the plants are yet to be scientifically proven for their therapeutic efficacy. The antidiabetic activity was not scientifically validated on seeds.

- *Delonix regia* (Boojer .Hook.) Raf., with agricultural and botanical limits, efforts were undertaken to unmask the extremely useful yet hidden therapeutic potential of the plant.
- The ethanomedicinal uses claimed were antidiarrhoeal, anti-inflammatory, hepatoprotective antioxidant, antimicrobial, anthelminitic, wound healing and gastroprotective activity and antidiabetic activity.

- No pharmacognostical and phytochemical work has been carried out on the seeds so far.
- The Antidiabetic activity was not yet scientifically validated.
- So the seed of the plant *Delonix regia* (Boojer .Hook.) Raf., was selected for evaluation of antidiabetic activity.

AIM AND OBJECTIVES

5. AIM AND OBJECTIVE

In the present scenario many countries in the world started giving importance for their own traditional and tribal medicines. The available treatment is only palliative and need to be taken almost life long.

Considering the side effects and other complications caused by the modern drug therapy, natural medicine is attracting increasing number of people.

AIM

The present study is to explore the Pharmacognostical, Phytochemical and Pharmacological studies on the seeds of *Delonix regia* (Boojer .Hook.) Raf.

OBJECTIVE

- Collection and authentication of the plant specimen.
- Establishing the pharmacognostical profile of the plant.
- Extraction of plant materials, to prepare various extracts of *Delonix regia* (Boojer .Hook.) Raf., seeds (hexane, ethyl acetate, ethanol, water).
- Phytochemical screening of the crude powder and various extracts.
- To evaluate an *in vitro* antioxidant activity to identify the active extract.
- To evaluate the *in vitro* & *invivo* antidiabetic activity of the active extract.
- To isolate the active constituent present in the active extract using column chromatography.
- To characterize the isolated compounds by spectral studies like IR and GC – MS spectroscopy.

PLAN OF WORK

6. PLAN OF WORK

PHARMACOGNOSTICAL STUDIES

- Collection and Authentication of plant material.
- Macroscopy.
- Microscopy.
- Powder microscopy.
- Linear measurement.
- Histochemical studies.
- Physicochemical constants.
- Qualitative analysis of heavy metals and inorganic elements.
- Quantitative estimation of heavy metals and inorganic elements.

PHYTOCHEMICAL STUDIES

- Preparation of extracts.
- Fluorescence analysis of powder and extracts.
- Preliminary phytochemical screening of powder and extracts.
- Quantitative estimation of phytoconstituents.
- Thin layer chromatography of extracts.
- High performance Thin Layer Chromatography of extracts.

PHARMACOLOGICAL STUDIEDS

- *In vitro* studies selection of active extract.

    a. *In vitro* Antioxidant activity.

        1,2 – Diphenyl – 2- Picryl Hydrazyl radical scavenging assay.

    b. *In vivo* Antidiabetic activity.

        α-Amylase Inhibition assay.

- *In vivo* evaluation of Antidiabetic activity.
    a. Acute toxicity study.
    b. Streptozotocin induced Hyperglycaemic.
    c. Collection of blood and organs.
    d. Histopathological study.
    e. Statistical analysis.

ISOLATION OF ACTIVE COMPOUNDS

- Isolation of compounds from active extract.
- Infra red spectroscopy of isolated compounds.
- GC – MS spectroscopy analysis of isolated compounds.

MATERIALS AND METHODS

7. MATERIALS AND METHODS

PHARMACOGNOSTICAL STUDIES

Collection and authentication of plant material

The seeds of *Delonix regia* (Hook.) Raf., was collected from Nungambakkam, Chennai-03, Tamil Nadu in July-2015. The plant material was confirmed and authenticated by Dr. M. Palanisamy, Scientist 'D'- In – Charge, Botanical Survey of India, Southern Regional Centre, T. N. A. U Campus, Lawley Road, Coimbatore-641003. The specimen copy of this plant is preserved in the Department of Pharmacognosy herbarium to future reference. The voucher number is 14/PCOG/2015.

MACROSOPY [75]

The plant material is categorized according to sensory characteristics. Organoleptic evaluation provides the simplest and quickest means to establish the identity, purity and quality of a particular sample. Hence this observation is of primary important before any further testing can be carried out.

MICROSCOPY

Staining method

a. Fixation of plant material[76]

The sample or seed was cut fixed in FAA solution (Formalin 5ml + Acetic acid 5ml + 90ml of 70% Ethanol). The specimen was dehydrated after 24 hours of fixing. The seeds were graded with series of tertiary butyl alcohol, as per the standard method.

b. Infiltration of the specimen[77]

It was carried out by gradual addition of 58 - 60°C of melting pointed paraffin wax until Tertiary Butyl Alcohol (TBA) solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning[78]

The paraffin embedded specimens were sectioned with the help of Rotary microtome. The thickness of the sections was 10 - 12µ. Dewaxing of the sections was done by customary procedures. The sections were stained Toludine blue. Since toludine blue is a polychromatic stain, the sections were stained as per the method published by O'Brein *et al*. The staining results were remarkably good. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to subrein, violet to mucilage and blue to the protein bodies. Whenever necessary sections were also stained with safranin, fast green and iodine for starch.

PHOTOGRAPHS

Microscopic descriptions were supplemented with photo micrographs whenever necessary. Photographs of different magnifications were taken with Samsung, lab photo two microscopic units. For normal observations bright field was used.

For the study of crystals and lignified cells, polarized light was employed. Since, these structures have birefringent property under polarized light they appear bright against

dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books.

POWDER MICROSCOPY [79-81]

The shade dried, powdered plant material was used for powder microscopic analysis. The Organoleptic characters were observed and to identify the different characteristic features various staining reagent were used. Powder was stained with 1% phloroglucinol in 90% ethanol, concentrated hydrochloric acid and observed through microscope. All the lignified cells stained with pink colour.

HISTOCHEMICAL STUDIES [82]

Portions of fresh seeds of the plant of *Delonix regia* (Boojer .Hook.) Raf., was used. The seeds were soaked in Formalin, Acetic acid, Ethanol before taking the section. The sections were stained using specific reagents (N/50 Iodine, dilute ferric chloride, phloroglucinol and concentrated hydrochloric acid. Picric acid, Dragendroff's reagent and O- toludine blue) to observe and locate starch, lignin, tannin, protein, alkaloid and flavonoid respectively as per the protocols. The stained sections were then washed in water to remove the excess stain and observed under a microscope (Magnus). Pictures of the sections were taken by Samsung camera fixed with Magnus microscope and processed by using the software Photoshop.

PHYSIO – CHEMICAL EVALUATION [82, 83-85]

Shade dried powdered plant material of seeds of *Delonix regia* (Boojer .Hook.) Raf,. was used for the determination of physio-chemical constants in accordance with WHO guidelines.

DETERMINATION OF ASH VALUES

Ash values are helpful in determination the quality and purity of a crude drug in the powdered form. The residue remaining after incineration is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or deliberately added to it, as a form of adulteration.

TOTAL ASH

Total ash is designed to measure the total amount of material remaining after ignition. This includes both physiological ash which is derived from plant tissue itself and non – physiological ash which is the residue of the extraneous matter adhering to the plant surface.

Procedure

Silica crucible was heated to red hot for 30 minutes and it was allowed to cool in desiccators. About 2gm of powdered sample was weighed accurately and evenly distributed in the crucible. Dried at 100 - 105°C for 1 hour and ignited to constant weight in a muffle furnace at 600 ± 25°C. The crucible was allowed to cool in desiccators. The percentage of ash with reference to the air dried substance was then calculated by the formula.

$$\%\, Total\ ash\ value = \frac{Wt.of\ Total\ ash}{Wt.of\ crude\ drug\ taken} \times 100$$

Water soluble ash

The ash was boiled for 5 minute with 25 ml of distilled water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of the ash and the difference in weight represented the water soluble ash, the percentage of water soluble ash with reference to the air dried substances was calculated by the formula.

$$\%\, Water\ soluble\ ash\ value = \frac{Wt.of\ Total\ ash\ - Wt.of\ Water\ soluble\ ash}{Wt.of\ crude\ drug\ taken} \times 100$$

Sulphated ash

2 – 3 gm of air dried substance was ignited gently at first in a crucible, until the substance was thoroughly charred. Then the residue was cooled, moistened with 1ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800 ± 25°C, until all the black particles were disappeared. The crucible was allowed to cool, a few drops of sulphuric acid was added and heated. Then it was ignited as before, cooled and weighed. The percentage of sulphated ash with reference to the air dried substance was then calculated.

Determination of extractive values

Extractive values are useful for the evaluation of phyto constituents especially when the constituents of a drug cannot be readily estimated by other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of water soluble extractives

5gm of the powder drug was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. 2ml of alcohol was added to the residue and it was dried at 105°C for1 hour in the hot air oven and cooled in desiccators for 30 minute and weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value with reference to the air dried drug was calculated.

$$Water\ soluble\ extractive\ value = \frac{Wt\ of\ dried\ extract}{Wt\ of\ sample\ taken} \times 100$$

Determination of alcohol soluble extractive

5gm of the powder was weighed and macerated with 100ml 90% ethanol in a closed flask for 24hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105°C for 1 hour in a hot air oven. The dish was cooled in desiccators and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air dried drug was calculated.

$$Alcohol\ soluble\ extractive\ value = \frac{Wt.of\ dried\ extract}{Wt.of\ Sample\ Taken} \times 100$$

Determination of ether soluble extractive

Transfer 5g of dried powdered drug to an extraction thimble and extract with solvent ether or petroleum ether Boiling Point 40-60ºc in a soxhlet for 6 hrs. The extract was filtered quantitatively into a tarred evaporating dish, evaporated and dried at 105ºc to constant weight. The percentage of ether soluble extractive value with reference to the air dried drug was calculated.

Determination of moisture content (Loss on Drying)

Specified quantity of the substances was taken in a previously ignited and cooled silica crucible and the substance was evenly distributed by gentle side wise shaking. The crucible with the contents was weighed accurately. The loaded crucible and the lid were placed in the drying chamber (105°C). The substance was heated for a specified period of time to a constant weight. The crucible was covered with the lid and allowed to cool in a desiccator at room temperature before weighing. Finally the crucible was weighed to calculate the loss on drying with reference to the air dried substance.

$$\%\ Loss\ on\ drying = \frac{Loss\ in\ weight\ of\ the\ sample}{Wt.of\ the\ sample} \times 100$$

Determination of foaming index

1gm of the coarsely powdered drug was weighed and transferred to 500ml conical flask containing 100ml boiling water. The flask was maintained at temperature 80 - 90°C for about 30min. it was then cooled and filtered into a volumetric flask and sufficient water was added through the filtrate to make up the volume to 100ml. the decoction was poured into 10 stopper test tube (height 16cm, diameter 16mm) in successive portions of 1ml, 2ml,

3ml, 4ml up to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml and the volume of the liquid in each tube was adjusted with water to 10ml. the tube were stopper and shake in a length of the foam was measured. The results are assessed as follows.

If the height of the foam in every tube is less than 1cm, the foaming index is less than100. If a height of 1cm is measured in any tube, the volume of the plant material decoction in the (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

If the height of the foam is more than 1cm in every tube, foaming index is over 1000. In this case repeat the determination using a new series of dilution of the decoction in order to obtain a result. Calculate the foaming index using the following formula:

$$\text{Foaming index} = 1000/a$$

Where, a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed.

Determination of swelling index

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant material were previously reduced to the required fineness was accurately weighed and transferred into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in an upward direction. Unless otherwise indicated in the test procedure,

add 25ml of water and shake the mixture thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. Calculate the mean value of the individual determination, related to 1gm of plant material.

QUALITATIVE AND QUANTITATIVE ANALYSIS OF HEAVY METALS AND INORGANIC ELEMENTS [86-89]

Plant minerals play a vital role in metabolism. Presence of elements vary with the soil, climate conditions etc. There are essential and non-essential elements which may be beneficial or harmful to living things. Non essential elements like lead, arsenic, cyanide, chromium, cadmium, aluminium, silver bring about toxic effects resulting in intoxification.

QUALITATIVE ANALYSIS OF INORGANIC ELEMENTS AND HEAVY METALS

To the ash of the drug material 50%v/v hydrochloric acid was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

Aluminium: White gelatinous precipitate of aluminium hydroxide is formed on addition of ammonia solution. It is slightly soluble in excess of the reagent. The precipitate dissolves readily in strong acid and base, but after boiling it becomes insoluble.

Arsenic: Arsenious salts in neutral solution react with solution of copper sulphate to form green precipitate (scheele's green) which on boiling gives a red precipitate of cupric oxide.

Borate: The mixture obtained by the addition of sulphuric acid and alcohol (95%) to a borate when ignited, burns with flame tinged with green.

Calcium: Solution of calcium salts, when treated with ammonium carbonate solution, yield a white precipitate after boiling and cooling the mixture (it is insoluble in ammonium chloride solution).

Carbonate: Carbonate, when treated with dilute acid effervescence, liberating carbon dioxide which is colourless and produces a white precipitate in calcium hydroxide solution.

Chlorides: Chlorides, when treated with silver nitrate solution, yield a white crude precipitate which is insoluble in nitric acid, but soluble after being well washed with water, in diluted ammonia, from which it is re precipitated by the addition of nitric acid.

Copper: An excess of ammonia, added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue coloured solution.

Iron: Solution of ferric salts, when treated with potassium ferrocyanide solution, yields an intense blue precipitate which is insoluble in dilute Hcl.

Lead: Strong solution of lead salts, when treated with Hcl, yield a white precipitate. Which is soluble in boiling water and is re deposited as crystals when the solution is cooled.

Magnesium: Solution of magnesium salts, when treated with ammonium carbonate solution and boiled, yield a white precipitate, but yield no precipitate in the presence of ammonium chloride solution.

Mercury: Solution of mercury salts, when treated with sodium hydroxide solution, yields a yellow precipitate.

Nitrate: With solution of ferrous sulphate no brown colour was observed but if sulphuric acid is added (slow from the side of the test tube), a brown colour is produced at the junction of two liquids, indicating the presence of nitrates.

Phosphate: Solution of phosphate when treated with silver nitrate with dilute ammonia solution and in dilute nitric acid yield yellow precipitate of normal silver ortho phosphate (distinction from meta and pyrophosphate) solution.

Potassium: Moderately strong potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.

Silver: Solution of silver salts, when treated with potassium iodide solution yield a cream coloured precipitate which is insoluble in dilute ammonia solution and in nitric acid.

Sulphates: Solution of sulphates, when treated with lead acetate solution yields a white precipitate which is insoluble in ammonium acetate solution and in sodium hydroxide.

QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS

Inductive coupled plasma-Optical emission spectroscopy (ICP-OES)

It is an excellent multi-element technique with relatively good sensitivity and selectivity when configured correctly. This technique utilizes the plasma as an ion source or light emission source are capable of producing values.

QUANTITATIVE ANALYSIS OF HEAVY METALS

Instrumentation parameters:

Instrument name: Inductive coupled plasma-Optical emission spectroscopy

Instrument Model: PE Optima 5300DV ICP-OES; Optical system Dual view-axial or radial

Detector system: Charge coupled detector, (UV-Visible detector which is maintaining at -40°C) to detect the intensity of the emission line.

Light source (Torch): Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2.0mm inner diameter.

Spray chamber: Scott type

Nebulizer: Cross flow gem tip.

Preparation of sample by acid digestion method:

50mg of powder was treated with acid mixture of sulphuric acid: water in the ratio of 4:1in the Kjeldahl flask and heated continuously till the solution is colourless. The sample mixture was then transferred in a 25ml volumetric flask and made up to the volume with distilled water. Blank solution was prepared as above without sample.

The standards of Arsenic, Lead, Mercury and Cadmium were prepared as per the protocol and the calibration curve was developed for each of them.

Detection:

Samples were analyzed for the detection and quantification of the aluminium, calcium, chloride, borate, silver, copper, potassium, magnesium, iron by Inductively Coupled Plasma Emission Spectrometry.

PHYTOCHEMICAL STUDIES

Phytochemical evaluation is used to determine the nature of phyto constituents present in the plant by using suitable chemical tests. It is essential to study the pharmacological activities of the plant. It can be done by confirmation with different chromatographic techniques like TLC and HPTLC. Therefore a complete investigation is required to characterize the Phytoconstituents qualitatively and quantitatively.

Preparation of Extracts[90]

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites into the extracting solvent depends upon its polarity.

Extraction

The first step was the preparation of successive solvent extracts. The dried coarsely powdered sample of *Delonix regia* (Hook.) Raf. Seed (500gm) was first extracted with Hexane in Soxhlet apparatus and then with solvents of increasing polarity like Ethyl acetate, ethanol (at 60 - 70°C) and water. Each extract was concentrated using rotary

vacuum evaporator. The percentage yield, colour and consistency of these extracts were recorded and preceded for further detailed phyto chemical and pharmacological screening.

PRELIMINARY PHYTOCHEMICAL SCREENING [91-103]

The chemical tests for various Phyto constituents in the dried powder and extracts of seeds of *Delonix regia* (Hook.) Raf., were carried out as described below and the results were recorded.

Detection of Alkaloids: Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the followings,

a. **Mayer's reagent** (Potassium mercuric iodide solution)

   Alkaloids give cream colour precipitate with mayer's reagent.

b. **Dragendorff's reagent (**Potassium bismuth iodide solution)

   Alkaloids give reddish brown precipitate with Dragendorff's reagent.

c. **Hager's reagent** (Saturated solution of picric acid)

   Alkaloids give yellow coloured precipitates with Hager's reagent.

d. **Wagner's reagent** (Solution of iodine in potassium iodide)

   Alkaloids give reddish brown precipitate with wagner's reagent.

Detection of Proteins

Biuret test: The sample was treated with 5-8 drops of 10% w/w copper sulphate solution, violet colour is formed.

Detection of Flavonoids

a. **Shinoda's test:** Small quantity was dissolved in alcohol to these pieces to magnesium followed by concentrated hydrochloric acid were added drop wise and heated. Appearance of magenta colour shows the presence of flavonoids.
b. **With aqueous sodium hydroxide solution:** Small quantity of the extract was dissolved in aqueous sodium hydroxide and appearance of yellow colour indicates the presence of flavonoids.
c. **Zinc hydrochloride test:** Small quantity the extract was mixed a mixture of zinc dust and conc. Hcl acid. It gives red colour after a few minutes.

Detection of Tannins

a. **Lead acetate test:** The test solution was mixed with basic lead acetate solution and examined for formation of a white precipitate.
b. **Ferric chloride test:** A few drops of 5% aqueous ferric chloride solution was added to 2ml of an aqueous extract of the drug and examined for the appearance of bluish black colour.

Detection of fixed oils and fats

a. **Spot test:** Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils and fats.
b. **Saponification test:** Few drops of 0.5 % alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hr. Formation of soap with the alkali indicates the presence of fixed oils and fats.

Detection of Glycosides

a. **Borntrager's test:** The powdered material was boiled with 1ml of sulphuric acid in a test tube for five minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammoniacal layer.
b. **Modified Borntrager's test:** The test material was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes, and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammoniacal layer.

Detection of Steroids and Triterpenoids

a. **Libermann Burchards test:** The powdered drug was treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added from the sides of the test tube; brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of tri terpenoids.
b. **Salkowski test:** The extract was treated with few drops of concentrated sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of tri terpenoids.
c. **Nollers test:** The extract was warmed with tin and thionyl chloride. Pink colouration indicates the presence of triterpenoids.

d. **Sulfur powder test:** The extract added with small amount of sulfur powder, it sinks at the bottom.

Detection of Carbohydrates

a. **Molisch's test:** To the test solution few drops of alcoholic solution of α napthol and few drops of conc. sulphuric acid were added through the sides of test tube, purple to violet colour ring appears at junction.
b. **Fehling's test:** The test solution was mixed with Fehling's I and II and heated and examined for the appearance of red coloration for the presence of sugar.

Detection of Saponins: A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3 minutes. Development of any honey comb like froth was examined.

FLUORESCENCE ANALYSIS [104-106]

Many crude drug show the Fluorescence when the sample is exposed to UV radiation. Evaluation of crude drugs based on fluorescence in day light is not much used, as it is usually unreliable due to the weakness of the fluorescent effect. Fluorescent lamps are fitted with suitable filter, which eliminate visible radiation from the lamp and transmits UV radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

TOTAL SAPONNIN CONTENT[107]

20 gm of the powder of seeds was put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hrs with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml of 20% of ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponins content was calculated as percentage.

TOTAL FLAVONOID CONTENT [108-109]

Total flavonoid content was measured by the aluminium chloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.30 ml of 5% $NaNO_2$, after 5min 0.3 ml of 10 % $AlCl_3$ was added. After 5min, 2 ml of 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

CHROMATOGRAPHY[109-110]

Chromatography methods are important analytical tool in the separation, identification and estimation of components present in the plant.

THIN LAYER CHROMATOGRAPHY

Principle

Thin layer chromatography is a technique used for the separation, identification and estimation of single or mixture of components present in the various extracts. It is reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase. The separation is mainly based on the differential migration that occurs when a solvent flows along the thin layer of stationary phase. This may be achieved by partition and adsorption depending on stationary phase used.

TLC Plate Preparation

The plates were prepared using Stahl TLC spreader. 40gm of silica gel G was mixed with 85ml of water to prepare homogenous suspension and poured in the spreader. 0.25mm thickness of plates was prepared, air dried until the transparency of the layer disappeared, then dried at 110°C for 30 minutes and kept in desiccators.

Selection of mobile phase

Solvent mixture was selected on the basis of the phyto constituents present in each extract. Factors such as nature of components, stationary phase, mobile phase, polarity, influence the rate of separation of constituents. From the vast analysis, best solvent was selected which showed good separation with maximum number of components.

The Retardation Factor (R_f) is calculated using following formula,

$$R_f = \frac{Distance\ travelled\ by\ solute\ from\ the\ origin}{Distance\ travelled\ by\ solvent\ from\ the\ origin}$$

HPTLC- FINGERPRINT PROFILE

HPTLC is one of the versatile chromatographic method which helps in the identification of compounds and thereby authentication of purity of herbal drugs. The time required in this method for the demonstration of most of the characteristic constituents of a drug is very quick and short. In addition to qualitative detection, HPTLC also provides semi- quantitative information on major active constituents of a drug, thus enabling an assessment of drug quality.

Instrument Conditions

Sample used **:** Ethanol Extract

Instrument **:** CAMAG HPTLC

HPTLC Applicator **:** CAMAG LINOMAT IV

HPTLC Scanner **:** CAMAG TLC SCANNER II

Sample dilution **:** 100mg of sample extracted with 1ml of Ethanol

Volume of injection **:** 20µl

Mobile phase **:** Hexane: Ethyl acetate: Chloroform: Methanol: Formic acid (2:3:5:0.5:0.5)

Lambda max **:** 400 - 800nm

Lamp : Tungsten

Stationary phase : TLC Aluminium coated silica gel 60 F254 (Merck)

Equipment: A Camag HPTLC system equipped with a sample applicator Linomat IV, Twin trough plate development chamber, TLC Scanner II.

Chromatographic conditions

The estimation has been done using the following chromatographic conditions. Chromatography was performed on a 12 × 3 cm (H x W) pre-activated HPTLC silicagel 60 F254 plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat IV with nitrogen flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner II. The plates were pre-washed with solvent ethyl acetate.

HPTLC serves as a convenient tool for finding the distribution pattern of phyto constituents which is unique to each plant. The fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extract.

PHARMACOLOGICAL STUDIES

STUDIES SELECTION OF ACTIVE EXTRACT

***IN VITRO* ANTI - OXIDANT ACTIVITY**[36]

DPPH assay: **(2, 2-diphenyl-1-picrylhydrazyl)**

0.1 ml of the chloroform extract was taken in test tubes. 6 ml of DPPH (diphenyl picryl hydrazyl) solution was added and the tubes kept in dark for one hour. The colour was read at 517 nm. The difference in the Optical density of DPPH solution and DPPH solution + sample was calculated. The decrease in OD with sample addition is used for calculation of the antioxidant activity. The activity was compared with BHT (butylated hydroxyl toluene) standard. Free radical scavenging activity was expressed as the inhibition percentage calculated using the formula

$$\%\ of\ Scavenging\ Activity = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

***IN VITRO* ANTI – DIABETIC ACTIVITY**[36,111]

α-amylase inhibition assay

In vitro amylase inhibition was studied by the method of Bernfeld. In brief, 100µl of the test extract was allowed to react with 200µl of α-amylase enzyme (Hi median Rm638) and 100µl of 2mM of phosphate buffer (P^H – 6.9). After 20 minute incubation, 100µ of 1% starch solution was added. The sample was performed for the controls where 200µl of the enzyme was replaced by buffer. After incubation for 5minutes, 500µl of dinitrosalicylic acid reagent was added to both control and test. They were kept in boiling

water bath for 5minutes. The absorbance was recorded at 540nm using spectrometer and the percentage inhibition of α-amylase enzyme was calculated using the formula

$$Inhibition\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Suitable reagent blank and inhibitor controls were simultaneously carried out.

ACUTE ORAL TOXICITY STUDY (UP AND DOWN METHOD) OECD 425 GUIDLINE FOR THE TESTING OF CHEMICALS [112]

The organization of economic co – operation and development (OECD) guideline 425 was followed the acute oral toxic up and down method is a stepwise procedure which 5 rats of single sex per step (one animal per step). Depending upon the mortality and morbidity status of the animal, on average of 2 to 4 steps may be necessary to allow judgement on the acute oral toxicity of the substance. This procedure results in the use of minimal number of animal while allowing for acceptable data based scientific conclusion.

Literature review showed that the *Delonix regia* is not toxic till the dose level of 2000mg/kg. Hence a starting dose level of 2000mg/kg of polysaccharides of *Delonix regia* seeds was used. After oral administration, animals were observed at an hourly basis for the first 4 hour and periodically for 24 hour to assess the general behaviour for 72 hours toxic symptoms and mortality of the animal for 28 days.

IN VIVO ANTI DIABETIC ACTIVITY [36, 114 - 118]

EVALUATION OF ANTI DIABETIC ACTIVITY STREPTOZOTOCIN INDUCED DIABETES

EXPERIMENTAL DESIGN

Plant extracts

i. Ethanol extract of *Delonix regia* (Hook.) Raf., seeds.

Chemicals

ii. Streptozotocin was obtained from Lab chemicals, India.

iii. Glibenclamide was obtained from Aventis pharmaceuticals, Goa.

Experimental animals

Healthy wistar albino male rats (weighing 170-230mg) were produced from Madras Medical College Animal house. The entire process was approved by the Institutional Animal Ethical Committee which is certified by the Committee for the purpose of control and supervision of experiments on animals, India (CPCSEA). 12/243/CPCSEA.

The animals were kept clean and dry polycarbonate cages and maintained in a well ventilated animal house with 12 hour light / 12 hour dark cycle. The animals were with standard pellet diet and water was given adlibitum. For experimental purpose the animals were kept fasting overnight but allowed for access to water.

EXPERIMENTAL INDUCTION OF NON – INSULIN DEPENDENT DIABETES MELLITUS (NIDDM)

The rats were injected intraperitoneally with Streptozotocin dissolved in citrate buffer (P^H 4.5) at a dose of 60mg/kg body weight. Hyperglycaemia was confirmed by the elevated blood glucose level in plasma, determine at the end of 0 hr, 1hr, 3hr, 5hr, 7hr, 24hr (Acute study Table 22)and then 7 day after injection. Rats with blood glucose level above 126mg/dl were selected for the study. Samples are analysed for blood glucose content by Glucometer.

EXPERIMENTAL DESIGN FOR STREPTOZOTOCIN INDUCED HYPERGLYCEMIC STUDIES

Animals were randomly divided into 5 group of 6 rats (n = 6)

Table 6. The animals received treatments

S. No	Group	Name of the drug	Dose	No of animals	Duration of dosage (days)
1	Group – 1	Normal control	Saline	6	28
2	Group – 2	Diabetic control (0.9% v/v saline)	2ml p.o	6	28
3	Group – 3	Standard (glibenclamide)	3mg/kg p.o	6	28
4	Group – 4	Test drug – I	200mg/kg p.o	6	28
5	Group – 5	Test drug – II	400mg/kg p.o	6	28

In an earlier study, the experimental animals were fasted for 18 hours and the Blood glucose level (BGL) was monitored using a Glucometer after Streptozotocin Injection. Blood samples were collected by tail clipping method. Rats with blood glucose level of greater than 250mg/dl were considered diabetic and were selected for study (WHO, 1985) Rats were divided randomly into 5 Groups of 6 rats per group for screening.

Streptozotocin monohydrate 60mg/kg body weight was dissolved in 0.9% v/v Cold normal saline and injected intraperitoneally to 18 hours fasted rats (24 no's,) group II-IV in order to induce hyperglycaemia in experimental Wistar rats (170–230 g body Weight (b/w) and the six control rats (group-I) received equal volume of 0.9% v/v Cold normal saline injected intraperitoneally.

BIOCHEMICAL ANALYSIS

ORAL GLUCOSE TOLERANCE TEST (OGTT)

Glucose 2g/kg fed 30 minutes prior to the administration of extracts. Blood withdrawn from retro orbital sinus at 30, 60 and 120 minutes and 1st, 2nd and 3rd week (Chronic study Table 23) then the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using GOD – POD kit. Other parameters like serum total protein, albumin, creatinine, urea, total bilurubin, SGOT, SGPT, ALP and Total cholesterol (TC), serum triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL) was estimated.

HYPOLIPIDEMIC ACTIVITY

Determination of lipid profile and lipoproteins

At the end of the study, blood sample were collected from retro orbital plexus into universal non – heparinised tubes. Allow it to clot in air, which was later centrifuged at 5000 rpm for 5minuts and serum separated to precede analysis. Initial and final body weights were analysed.

Serum levels of the following tests were determined by auto analyser in the biochemical laboratory, Madras Medical College, Chennai. Parameters like Total Cholesterol (TC), Serum Triglycerides (TG), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Very Low Density Lipoprotein (VLDL) was estimated.

Serum VLDL

The value of VLDL was calculated as follows

$$VLDL\ cholesterol = \frac{Triglycerides}{5}$$

Serum LDL

The value of LDL was calculated as follows

LDL cholesterol = Total cholesterol – (HDL cholesterol + VLDL cholesterol)

COLLECTION OF BLOOD AND ORGANS[112,114,118]

The treatment is carried up to 28 days and on 1st, 7th, 14th and 21st days 0.5 ml of blood was collected from lateral tail vein using lance or butterfly needle and blood glucose level was checked by using a Glucometer. After 28 days the blood was collected and used to determine haematological parameters. The test animals were anesthetized with ketamine

at the dose of 10 mg/kg and sacrificed. Pancreas was isolated and used for histopathological studies.

HISTOPATHOLOGICAL STUDY[112,114,118]

For histological examinations, small pieces of pancreas were fixed In Bouin's Solution for 24h dehydrated through graded concentration of ethanol, embedded in Paraffin wax, sectioned at 5µm thicknesses and stained with Mayer's haematoxylin and Eosin and observed under light microscope.

STATISTICAL ANALYSIS

Results were expressed as Mean ± S.E.M. The data was analyzed using One Way of Variable (ANOVA) followed by Dennett's test. P-value <0.05 considered as significant.

ISOLATION AND CHARACTERISATION OF PHYTOCONSTITUENTS BY COLUMN CHROMATOGRAPHY [100-103]

Column chromatography is an isolation technique in which phytoconstituents are being eluted by adsorption. The principle involved in this separation of constituents is adsorption at the interface between solid-liquid. The component must have various degree of affinity towards adsorbent and also reversible interaction to achieve successful separation. No two compounds are alike in above respect. Low affinity compound will elute first. Adsorbent used is silica gel.

- Ethanolic extract of *Delonix regia* (Hook.) Raf., seeds *was* found to posses maximum phytoconstituents with anti diabetic activity. An attempt was made to fractionate the ethanolic extract by column chromatography.

COLUMN CHROMATOGRAPHY

Type of extract : Ethanolic extract

Method : Dry packing method

Packing material : Silica gel G70-325

PROCEDURE

The ethanolic extract was subjected to Silica gel column chromatography for isolation of phytoconstituents.

An appropriate column sized 2.5 cm diameter and 60 cm length was used. It was washed with water and rinsed with acetone and dried completely. Little of pure cotton was placed at the bottom of column with help of a big glass rod. Solvent Hexane was poured in to the column $3/4^{th}$ level. Before packing, ethanolic extract 10 gm was diluted with same solvent and thoroughly mixed with 30 g of graded silica gel.

It was constantly mixed until it became free flow. When it reached at defined state it was slowly poured in to the column containing hexane solvent with slight movement of stirring by glass rod to avoid clogging. Once it got settled, little cotton was placed on top of silica gel extract mixture pack to get neat column pack.

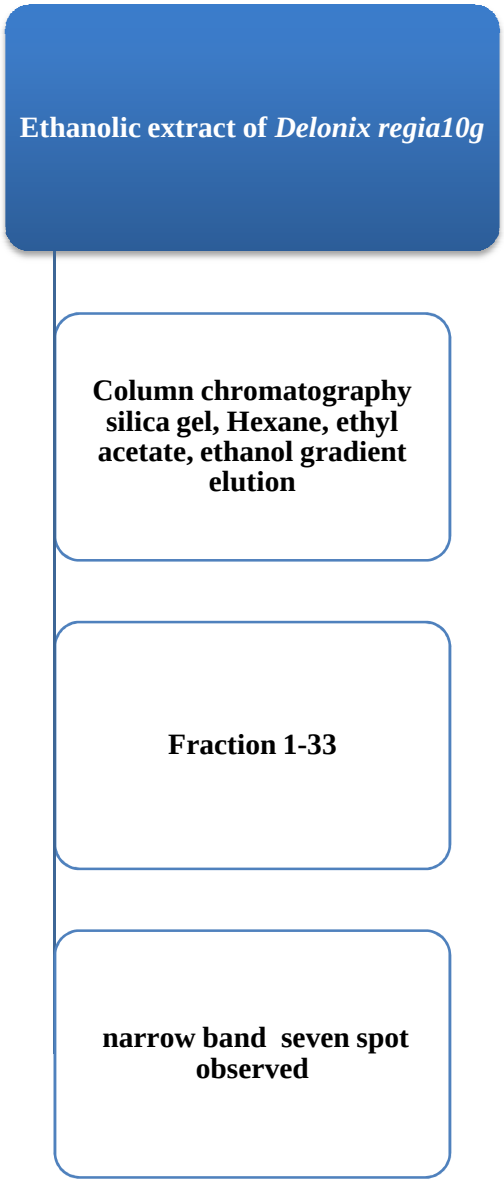

Fig .15 Method Column chromatography

The knob at bottom was slowly opened to release the solvent. The elute was done using hexane, followed by hexane-ethyl acetate mixture and ethyl acetate-ethanol mixture. Each fraction were collected and analysed quantitatively. Similar fractions were mixed together, allowed to evaporate to remove solvent.

THIN LAYER CHROMATOGRAPHY (TLC)

As soon as the fraction eluted, it was analyzed by using readymade TLC plate with suitable mobile solvent according to the polarity of elute.

SOLVENT SYSTEM

Ethanol: Ethyl acetate in 3.5: 2.5

OBSERVATION

The developed chromatogram was observed under UV, Fluorescence and also derivatised using detecting agent.

SPECTRLA ANALYSIS

Purified single isolated compound was taken to determine the structural by instrumental spectral analysis such as

a. IR spectroscopy
b. GC – MS spectroscopy

a. IR spectroscopy

IR spectrum is considered as vibrational – rotational spectra. KBR pellet technique was used for solid compound, for liquid compound Nujol mull method was followed. It is very helpful record which would give information about functional group present in the organic compounds. Mechanism of bond stretching and bending is happened when electromagnetic radiation ranging from $500cm^{-1}$ to $4000cm^{1}$ passed through sample. Instrument used was ABD BOWMAN spectrometer.

b. GC-MS

It is combined technique, used for molecular weight determination. Gas chromatography and mass spectroscopy combined to form GC – MS.

RESULTS AND DISCUSSION

8. RESULT AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

Organoleptic characters:

Nature	:	Soft and glossy
Colour	:	Brown
Odour	:	Odourless
Taste	:	Tasteless

Morphological characters:

Colour	:	Greenish brown
Appearance	:	Hard seed coat
Shape	:	Oblong
Seed length	:	2.3 – 2.6cm
Seed width	:	0.6 – 0.8cm
Surface	:	Smooth and shine
Thickness	:	1.2 – 1.8mm

8. RESULT AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

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Fig .16 *Delonix regia* (Boojer .Hook.) Raf. seeds

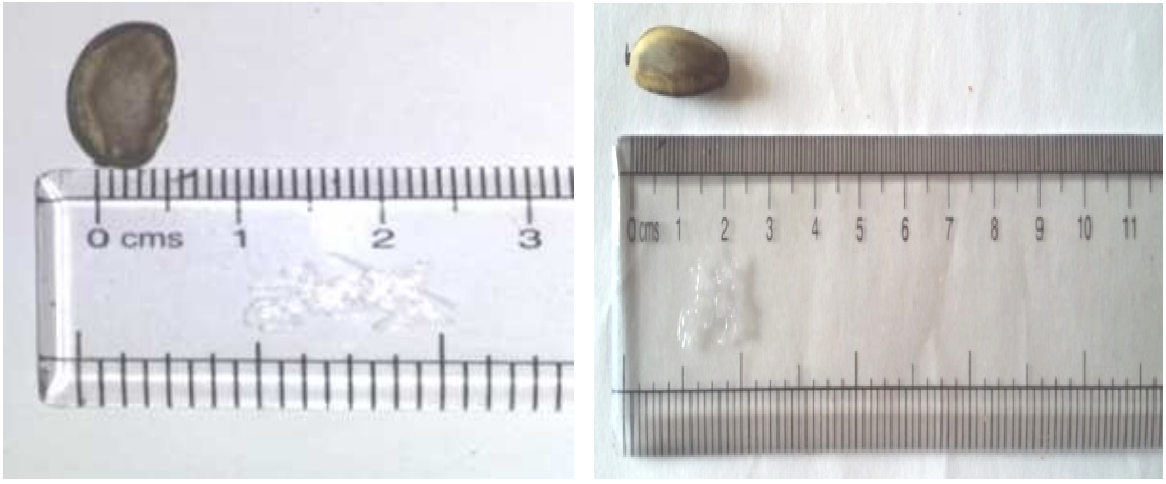

Fig .17 *Delonix regia* (Boojer .Hook.) Raf. seed length and width

Fig .18 *Delonix regia* (Boojer .Hook.) Raf. seed pods

Microscopical features

Transverse section of seed

- The seed coat is 1.2mm thick. It contains of outer epidermal layer followed by a thin zone of 2 or 3 years of parenchyma cells.
- The middle part of the seed coat consists of vertical layer elongated, compact sclereids.
- The sclerotic zone merges with a lignified inner seed coat include 3 or 4 layers of circular, compact parenchyma zone.
- The outer epidermal sub epidermal layers are 90µm thick. The outer sclerotic seed coat is 150µm thick.
- The inner sclerotic seed coat is 700µm thick. The inner most parenchymatous zone is 140µm thick.
- When viewed under polarized light the middle part of the seed coat which include outer and inner sclerotic layer appear bright indicating the presence of lignified walls.
- The cells of the inner and outer seed coat are vertically elongated narrow cylindrical cells. They have very narrow cell lumen.

MICROSCOPIC FEATURES OF THE SEEDS

TRANSVERSE SECTION OF THE SEED

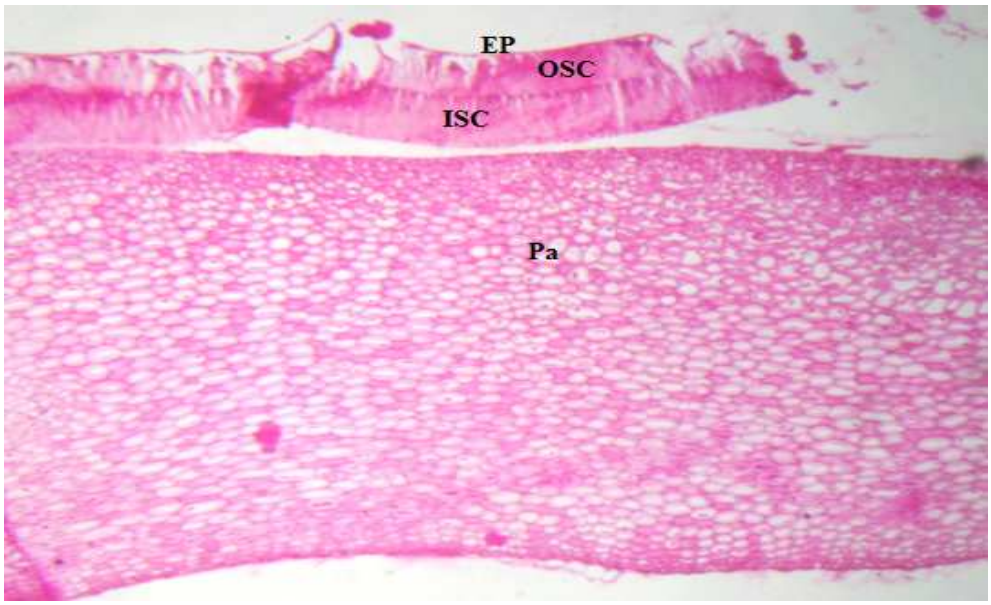

Fig 19. Seed Coat entire view

Fig 19. Seed Coat entire view

EP – Epidermis

OSC – Outer seed coat

ISC – Inner seed coat

Pa - Parenchyma

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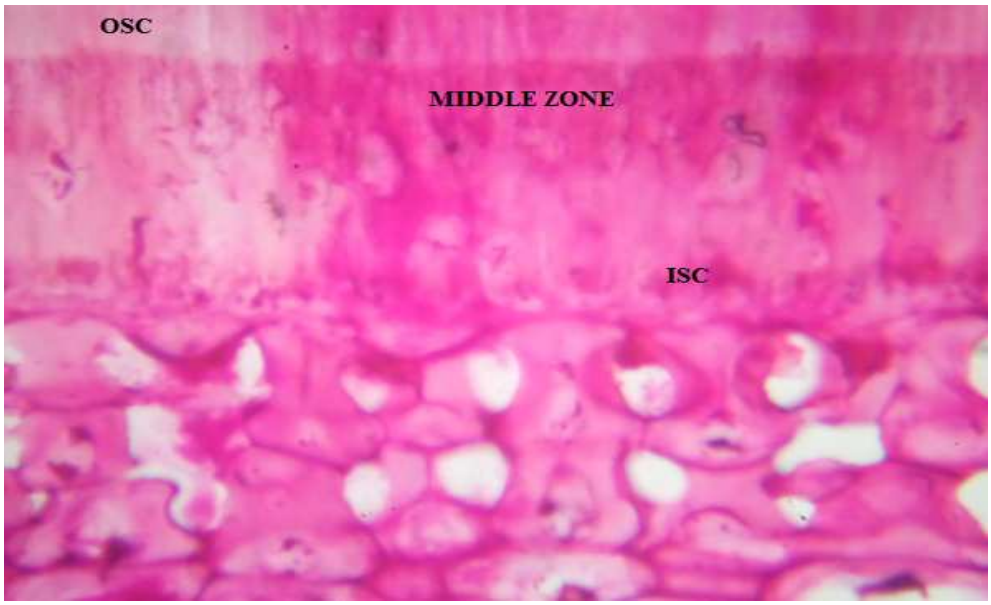


Fig 20. Middle sclerotic zone in transverse section

Fig 20. Middle sclerotic zone in transverse section

OSC – Outer seed coat

ISC – Inner seed coat

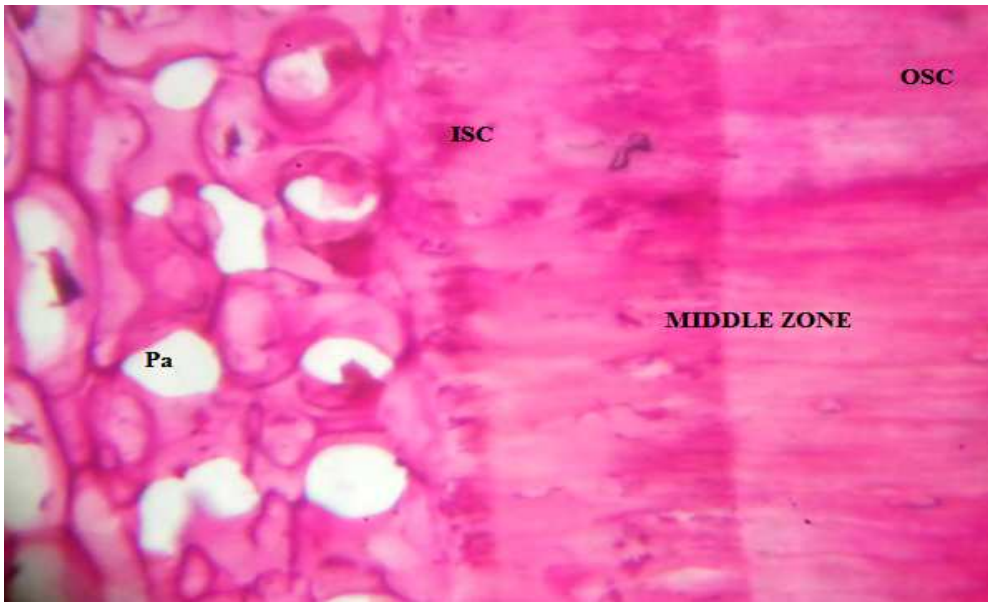


Fig 21. Seed coat showing inner seed coat and inner parenchyma zone

Fig 21. Seed coat showing inner seed coat and inner parenchyma zone

OSC – Outer seed coat

ISC – Inner seed coat

Pa – Parenchyma

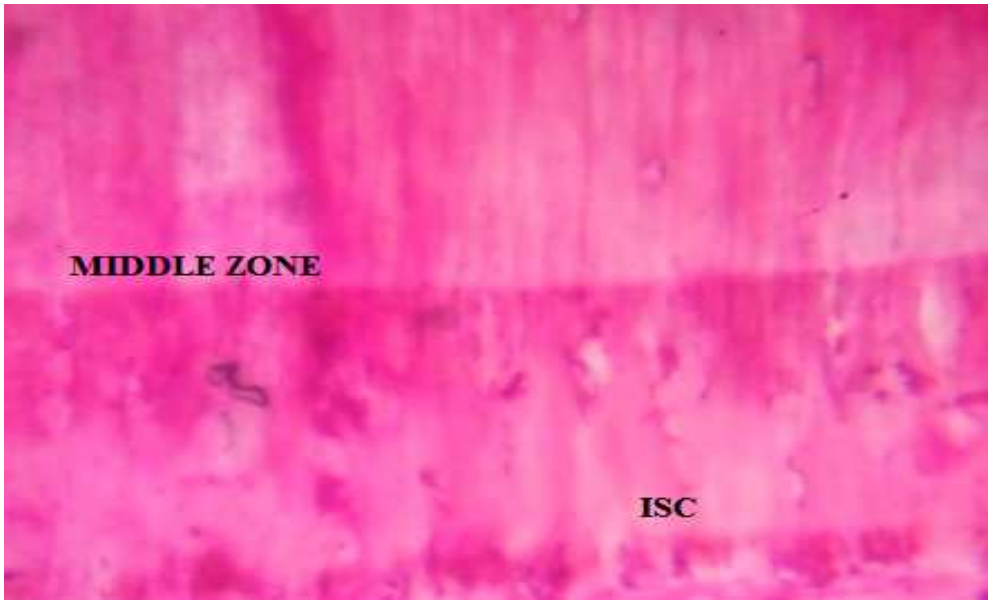


Fig 22. Inner sclerostesta enlarged

Fig 22. Inner sclerostesta enlarged

ISC –Iuter seed coat

POWDER MICROSCOPY

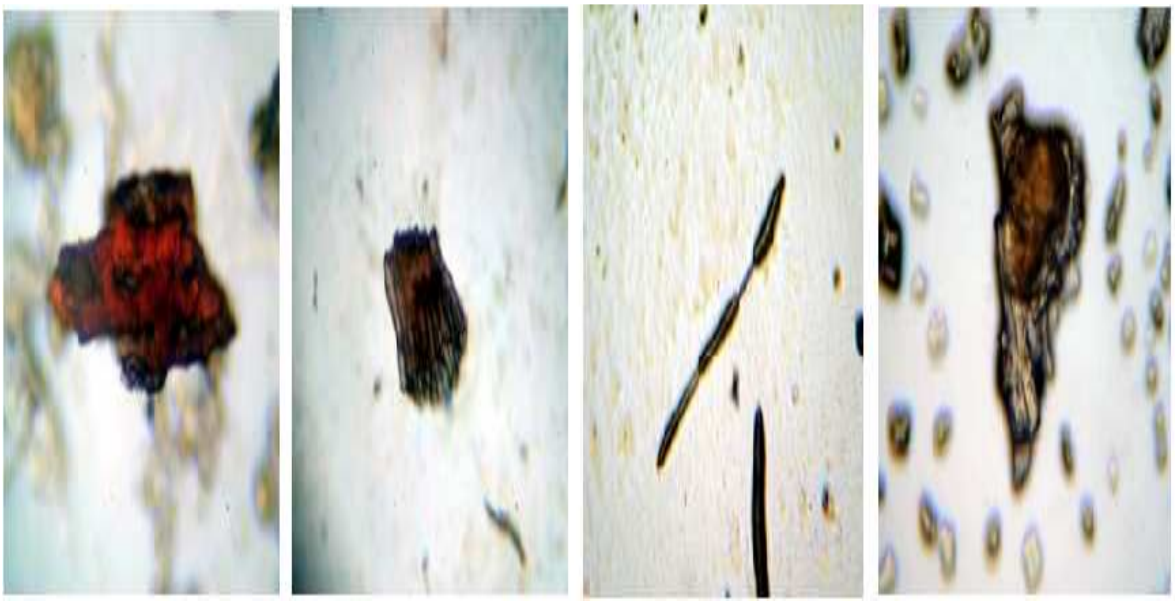
The powder preparation of the seed includes the following elements

- Thick pieces of sclerostesta are often visible in the powder. These pieces consist of compact vertically elongated columnar sclereids which are compactly arranged.
- When the columnar sclereids were separated the outer ends of the sclereids consists of thin long fibrils. The columnar sclereids are 250 µm long and 20 µm thick. The fibril portion is 100µ m long.
- Sclereids Circular branchy sclereids are often seen in the powder. They have very thick lignified walls and circular, wide lumen Fig. 22a. The sclereids are 200X120 µm in size.

- Sclerenchyma cells Small fragments of thick walled sclerenchyma cells are seen in Fig. 22c. The cells are elongated and rectangular with wide lumen .The cells are 50x 100µm in size.

a b c

a). Palisade epidermis, b).Parenchyma c). Sclerenchyma



d e f g

d). Sclerotic seed coat, e). Isolated bearer cells, f). Trichomes, g). Calcium oxalate crystals

Fig 23. Powder microscopical features

HISTOCHEMICAL COLOUR REACTION

Table 7. Histochemical colour reactions of seeds of *Delonix regia* (Boojer .Hook.) Raf.

S. No	Chemicals	Test for	Nature of change	Histology	Degree of change
1	Phloroglucinol + Hcl	Lignin	No pink colour	Middle seed coat	–
2	N/50 Iodine solution	Starch	No blue colour	Endosperm & embryo	–
3	Dil. Ferric chloride	Tannin	No bluish black colour	Epidermal layer & inner seed coat	–
4	Picric acid	Protein	No yellow colour	Endosperm	–
5	Dragendroff's reagent	Alkaloid	No orange colour	Inner seed coat	–

Note: (-) Indicates the absence

PHYSIO – CHEMICAL CONSTANTS

Table 8. The Physicochemical analysis of the seeds of *Delonix regia* (Boojer .Hook.) Raf.

Parameters		Results (%W/W)
Ash values	Total ash	2.53±0.005
	Sulphated ash	15.0±0.20
	Water soluble ash	1.1±0.01
	Acid insoluble ash	0.7±0.01
Loss on drying		6.5±0.15
Extractive values	Water soluble extractive	11.0±0.1
	Alcohol soluble extractive	5.0±0.2
	Ether soluble extractive	2.9±0.05
	Non – volatile ether soluble extractive	3.6±0.05
Other parameters	Foaming index	<100
	Swelling index	NIL

Values are expressed as mean ±SD, n= 3

INORGANIC ELEMENTS AND HEAVY METAL ANALYSIS

Table 9. Qualitative analysis of inorganic elements of *Delonix regia* (Boojer .Hook.)

S. No	Inorganic elements	Observations
1	Aluminium	+
2	Chloride	+
3	Copper	+
4	Calcium	+
5	Iron	+
6	Borate	+
7	Potassium	+
8	Silver	+
9	Phosphate	-
10	Nitrate	-
11	Sulphate	-

+indicates presence, -indicates absence

QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS

Table. 10. Quantitative analysis of inorganic elements of *Delonix regia* (Boojer .Hook.)

S. No	Inorganic elements	Total amount (%W/W)
1	Aluminium	0.028
2	Chloride	0.052
3	Copper	0.009
4	Calcium	0.010
5	Iron	0.028
6	Borate	0.005
7	Potassium	0.020
8	Silver	0.007

QUANTITATIVE ESTIMATION OF HEAVY METALS BY ICP OES METHOD

The quantification of the individual heavy metals was analyzed for the powdered mixture of *Delonix regia* (Boojer .Hook.) Raf., by ICP-OES technique the following metals like arsenic, lead, cadmium were detected and quantified, results are given in the following table.

Table 11. Quantitative estimation of Heavy metals for *Delonix regia* (Boojer .Hook.) Raf.,

S. No	Results	Specification as per	WHO guidelines
1	Mercury	Not detected	Not more than 0.5ppm
2	Arsenic	0.053	Not more than 5.0ppm
3	Lead	0.042	Not more than 10ppm
4	Cadmium	0.002	Not more than 0.3ppm

The above observation showed that the material is within the limits as per WHO standard and it is safe to consume internally.

PHYTOCHEMICAL STUDIES

Table 12. Percentage yield of successive solvent extraction of various extracts of seeds of *Delonix regia* (Boojer .Hook.)

S. No	Extract	Method of Extraction	Physical Nature	Colour	Yield (%W/W)
1	Hexane	Successive solvent extraction	Semi-solid	Green	0.6
2	Ethyl acetate		Sticky	Brownish green	1.3
3	Ethanol		Semi-solid	Green	4.6
4	Aqueous		Solid	Brown	5.7

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Table 13. Qualitative Phytochemical analysis *Delonix regia* (Boojer .Hook.) Raf.,

Phytoconstituents	Powder	Hexane extract	Ethylacetate extract	Ethanol extract	Aqueous extract
Alkaloids	-	-	-	-	-
Saponins	+	+	-	+	+
Glycoside	-	-	-	-	-
Carbohydrates	-	-	+	+	-
Tannins and phenolic compounds	-	-	-	-	-
Flavonoids	+	-	+	+	-
Phytosterols	+	+	-	+	-
Proteins and aminoacids	-	-	-	-	-
Triterpenoids	+	+	-	+	-
Fixed oils and fats	+	+	-	+	-
Gums and mucilage	-	-	-	-	-

Note: (+) indicates presence, (-) indicates absence.

1.1.1. QUANTITATIVE ESTMATION OF PHYTOCONSTITUENTS

The *Delonix regia* (Boojer .Hook.) was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total Saponins and Flavonoids content in the ethanol extract were decided to be taken as parameters. Samples were drawn from three random samples of *Delonix regia* (Boojer .Hook.) and the total Saponins and Flavonoids content present in them were estimated.

Table 14. Quantitative Estimation of Phytoconstituents

S. No	Parameters	Values (µg/ml)
1	Total saponins	5.24 ±0.15
2	Total flavonoids	7.86 ± 0.65

FLUORESCENCE ANALYSIS

Table 15. Fluorescence characteristic of powdered samples of *Delonix regia* (Boojer .Hook.) at short UV and long UV.

Treatment	Day light	UV light	
		254nm	365nm
Powder as such	Brownish green	Dark brown	Pale brown
Powder + water	Pale brown	Dark brown	Pale brown
Powder + NaOH	Brownish green	Dark green	Pale brown
Powder + Hcl	Dark green	Brownish green	Pale brown
Powder + Acetic acid	Pale brown	Dark brown	Pale brown
Powder + Alc.NaOH	Brownish green	Dark green	Pale brown
Powder + Picric acid	Brownish yellow	Brownish yellow	Yellowish brown
Powder + Sulphuric acid	Pale brown	Brownish green	Pale brown
Powder + Nitric acid	Brownish green	Dark brown	Pale brown
Powder + Iodine	Dark brown	Dark brown	Dark brown

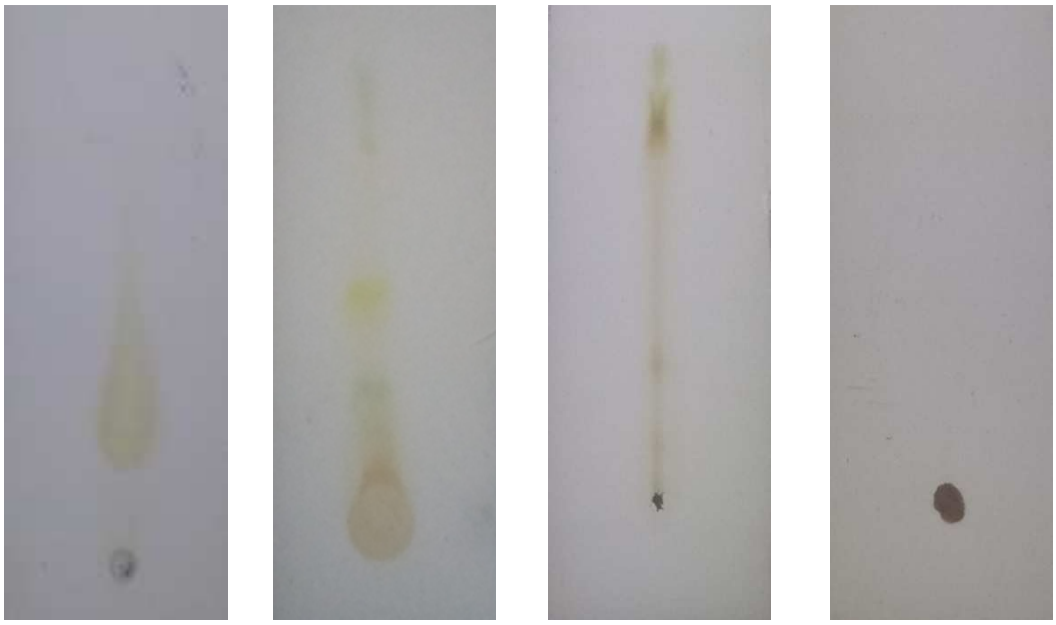
Table 16. Fluorescence analysis of various extracts of *Delonix regia* (Boojer .Hook.)

Treatment	Day light	UV light	
		Short UV(254nm)	Long UV (365nm)
Hexane extract	Dark greenish brown	Dark brown	Dark brownish
Ethyl acetate extract	Dark brown	Dark brownish green	Reddish brown
Ethanol extract	Dark brown	Dark brown	Reddish brown
Aqueous extract	Dark brown	Dark brown	Dark greenish brown

THIN LAYER CHROMATOGRAPHY OF EXTRACTS

Table 17. Thin layer chromatography of various extract of *Delonix regia* (Boojer .Hook.)

S. No	Extracts	Solvent system	No of spots	R_f value
1	Hexane	Ethyl acetate: Ethanol: Water 100:13.5:10	1	0.38 0.53 0.60
2	Ethyl acetate	Ethyl acetate: Ethanol: Water 100:13.5:10	3	0.55
3	Ethanol	Ethyl acetate: Ethanol: Water 100:13.5:10	2	0.59 0.80
4	Aqueous	Ethyl acetate: Ethanol: Water 100:13.5:10	-	-



a) b) c) d)

a)Hexane, b)Ethyl acetate, c)Ethanol, d)Aqueous

Fig 24. Thin Layer Chromatography of *Delonix regia* (Boojer .Hook.). Raf., seeds various extracts

HPTLC FINGER PRINT PROFILE

HPTLC Finger print Data of Ethanolic Extract of *Delonix regia* (Boojer .Hook.). Raf., High performance thin layer chromatography (HPTLC) finger printing was performed with the ethanol extract of *Delonix regia* (Boojer .Hook.) The chromatographic conditions were carried as detailed in material and method of this study. There were 5 peaks observed with different Rf Values and different heights. Percentage of areas was also obtained from the chromatogram.

SOLVENT SYSTEM

Table 18. Solvent system of HPTLC for EEDR

Extract	Solvent system
Ethanol	Hexane: Ethyl acetate: Chloroform: Methanol: Formic acid (2:3:5:0.5:0.5)

Fig 25. HPTLC for EEDR

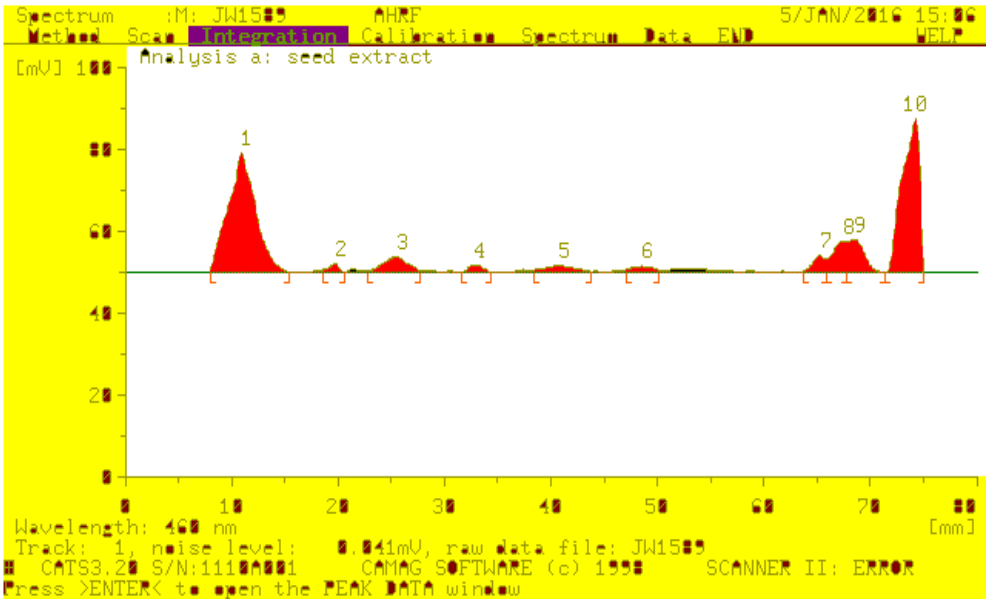


Fig 26. Chromatographic Finger Printing Analysis for EEDR

Table 19. HPTLC profile of EEDR

S. No	R_f	Height	Area (%)
1	0.11	29.1	861.9
2	0.20	2.3	23.7
3	0.25	3.7	100.2
4	0.32	1.8	27.0
5	0.41	1.8	55.2
6	0.48	1.5	32.1
7	0.65	4.1	53.7
8	0.67	7.6	111.2
9	0.68	8.0	144.0
10	0.74	37.7	701.2

PHARMACOLOGICAL STUDIES

SELECTION OF ACTIVE EXTRACT

RESULTS IN-*IN VITRO* ANTIOXIDANT ACTIVITY

DPPH assay (2, 2-DiPhenyl-1-PicrylHydrazyl)

Table 20. The % inhibition of radical scavenging activity

S. No	Concentration (µg/ml)	Standard BHT (butylated hydroxyl toluene)	Hexane Extract	Ethyl acetate Extract	Ethanol Extract	Aqueous Extract
1	200	33.62	19.12	24.33	30.97	14.16
2	400	39.31	23.59	29.62	36.09	18.21
3	600	85.97	35.22	62.47	79.75	22.69
4	800	90.53	42.01	68.92	82.68	30.19
5	1000	93.76	49.90	74.38	89.51	36.90
IC_{50} µg/ml		417	995	574.94	455	1474

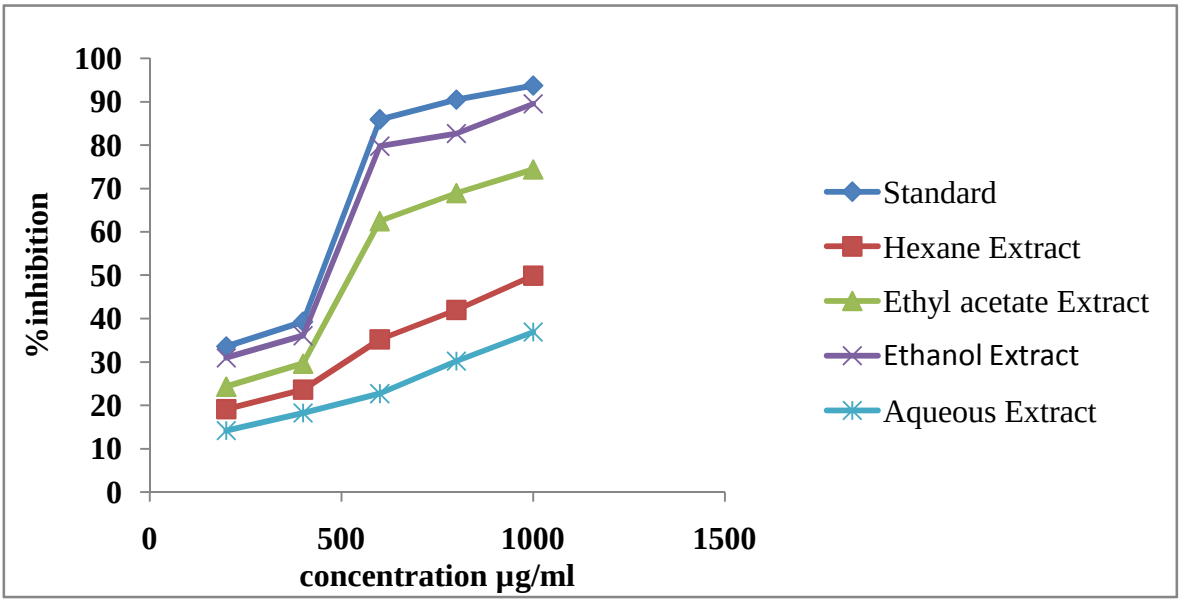


Fig 27. Graphical representation of the % inhibition of radical scavenging activity

RESULTS IN-*IN VITRO* ANTI DIABETIC ACTIVITY

α-AMYLASE INHIBITION ASSAY

Table.21. Results *in vitro* anti – diabetic activity

S. No	Concentration (µg/ml)	Standard (Acrabose)	Hexane extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1	200	31.53	12.35	22.34	27.94	9.07
2	400	67.38	14.07	28.02	48.05	12.01
3	600	72.49	16.38	34.32	57.04	14.36
4	800	78.53	30.46	42.27	63.08	16.28
5	1000	82.92	20.05	48.43	65.26	18.45
IC_{50}		374.436	1561	1049.19	550.29	3702

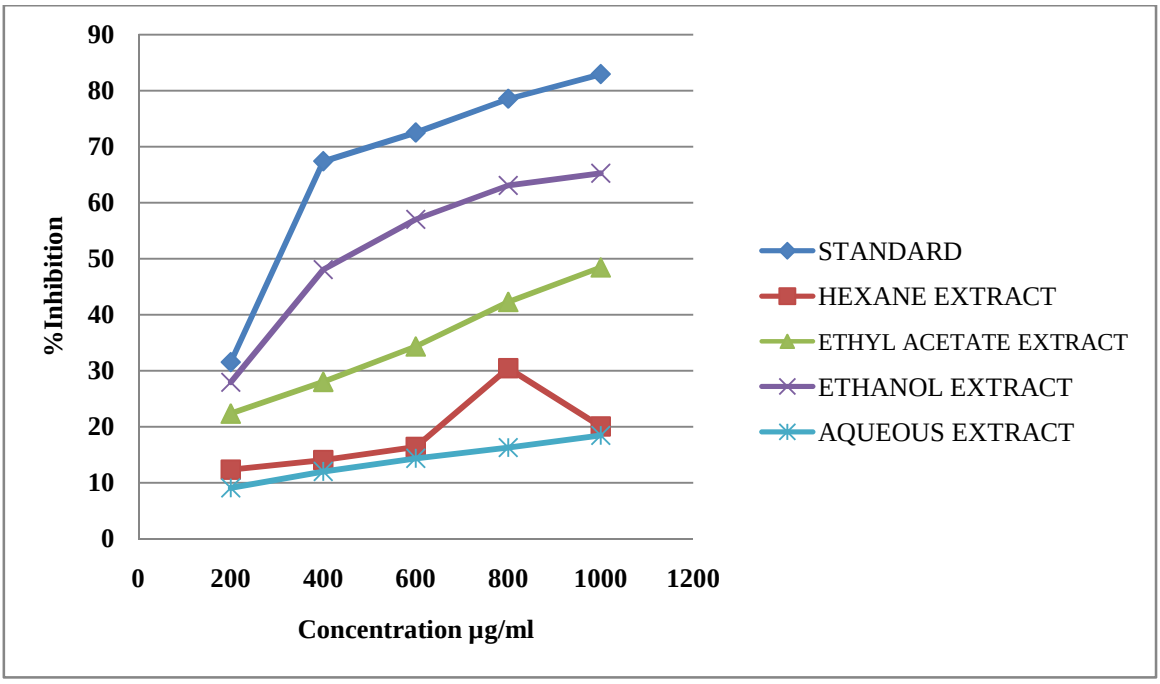


Fig 28. Graphical representation of the α-amylase inhibition assay

RESULTS IN-*IN VIVO* ANTIDIADETIC ACTIVITY

Effect of ethanolic extract of *Delonix regia* (Boojer .Hook.). Raf., on blood glucose level

Various studies show that a number of plant extracts effectively reduced the blood glucose levels in streptozotocin induced rats. Till date, the antidiabetic activity of seeds of *Delonix regia* (Boojer .Hook.). Raf., has never been reported.

Acute study

The antidiabetic effect of ethanolic extract at the dose of 200mg/kg and 400mg/kg produced a dose dependent hypoglycaemia on streptozotocin induced diabetic rats. It is clear from the data that the blood glucose levels of control diabetic animals continued to increase whereas extract treated diabetic rats showed significantly reduced levels.

Maximum reduction of blood glucose level was achieved by the ethanolic extract, 400mg/kg from 0 hours to 24 hours respectively, whereas ethanol extract at the dose of 200mg/kg showed a slightly lower effect.

Table 22. Effects of EEDR On blood glucose level in streptozotocin induced diabetic rats (mg/dl)-acute study.

Treatment	0 hr	1st hr	3rd hr	5th hr	7th hr	24th hr
Group I	83±1.3	93±1.6	102±1.2	112±1.8	99±1.1	101±1.9
Group II	372±0.4	374±1.1	385±0.2	390±1.5	407±1.8	431±2.1
Group III	342±1.2	315±1.4	288±1.7	246±2.3*	225±1.2*	198±1.8*
Group IV	352±2.1	348±2.2	332±2.1	276±1.3*	252±1.6*	232±1.6*
Group V	334±1.4	329±0.8	297±1.3	262±2.3*	233±2.2*	210±2.3*

One way ANOVA values are expressed as mean ±SD n = 6

*P < 0.05 compared to diabetic control

Note:

- ❖ Group I Normal control
- ❖ Group II Diabetic control
- ❖ Group III Standard drug (Glibenclamide 3mg/kg)
- ❖ Group IV Ethanolic extract of *Delonix regia* 200mg/kg
- ❖ Group V Ethanolic extract of *Delonix regia* 400mg/kg

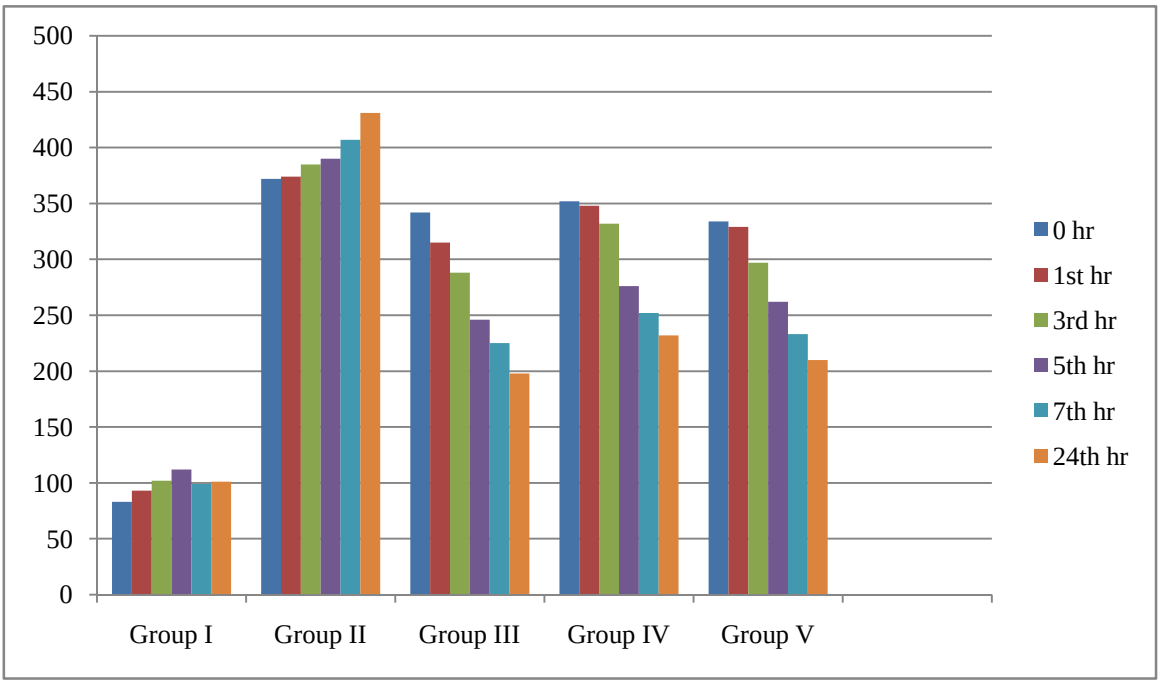


Fig 29. Graphical representation of blood glucose level in study groups-acute study

- ❖ Group I compared with Groups II, III, IV, V
- ❖ Group II compared with Groups III, IV, V

Chronic study

Chronic administration of *Delonix regia* (Boojer .Hook.). Raf., extracts to streptozotocin induced diabetic rats for 28 days produced a significant blood glucose reduction. A significant reduction was observed right from 1st week by ethanolic extract which was comparable with that standard drug, Glibenclamide (3mg/kg).

Table 23. Effects of EEDR On blood glucose level in streptozotocin induced diabetic rats (mg/dl)-chronic study.

Treatment	0 day	7th day	14th day	21st day	28th day
Group I	90±1.4	96±1.3	100±1.2	99±1.2	93±1.7
Group II	264±1.4	278±1.6	284±0.8	290±1.1	307±1.9
Group III	250±0.9	248±0.5	200±1.3*	182±1.3*	148±1.2*
Group IV	278±1.3	274±2.7	220±1.9*	198±1.3*	187±1.5*
Group V	270±1.2	261±2.3	206±1.5*	186±2.5*	173±1.6*

One way ANOVA values are expressed as mean ±SD n = 6

*P < 0.05 compared to diabetic control

Note:

- Group I Normal control
- Group II Diabetic control
- Group III Standard drug (Glibenclamide 3mg/kg)
- Group IV Ethanolic extract of *Delonix regia* 200mg/kg
- Group V Ethanolic extract of *Delonix regia* 400mg/kg

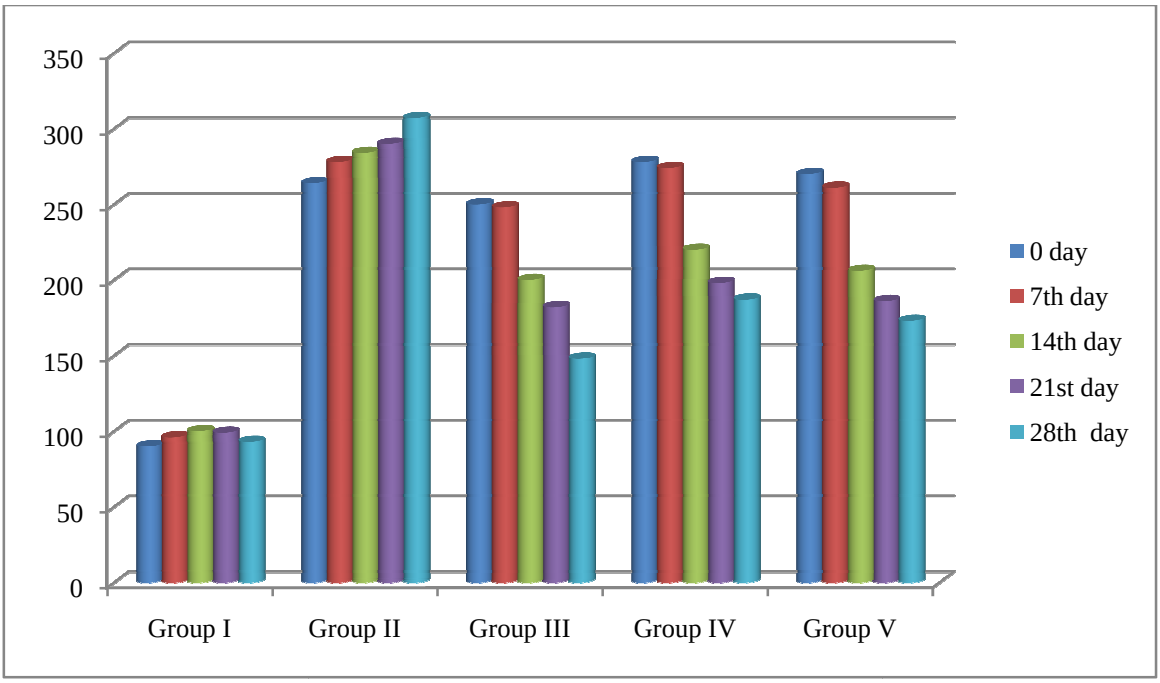


Fig 30. Graphical representation of blood glucose level in study groups-chronic study

- ❖ Group I compared with Groups II, III, IV, V
- ❖ Group II compared with Groups III, IV, V

Changes in Body weight

Table 24. Changes in Body Weight (grams)

Treatment	0 day	7th day	14th day	21st day	28th day
Group I	121±6.68	125.67±6.68	131.67±6.68	123.67±6.68	127.67±6.68
Group II	127.12±5.51	133±6.33	147.38±3.25	148.26±3.25	130.57±4.15
Group III	134.28±4.11	121.01±3.18	115.47±1.71*	109.19±6.15*	100.87±7.54*
Group IV	140.51±3.64	135.47±5.48	123.24±2.31*	112.35±8.48*	107.95±3.18*
Group V	147.17±9.47	129.17±3.49	120.54±5.47*	107.68±4.67*	103.91±1.26*

One way ANOVA values are expressed as mean ±SD (n = 6)

*P < 0.05 compared to diabetic control

Note:

- ❖ Group I Normal control
- ❖ Group II Diabetic control
- ❖ Group III Standard drug (Glibenclamide 3mg/kg)
- ❖ Group IV Ethanolic extract of *Delonix regia* 200mg/kg
- ❖ Group V Ethanolic extract of *Delonix regia* 400mg/kg

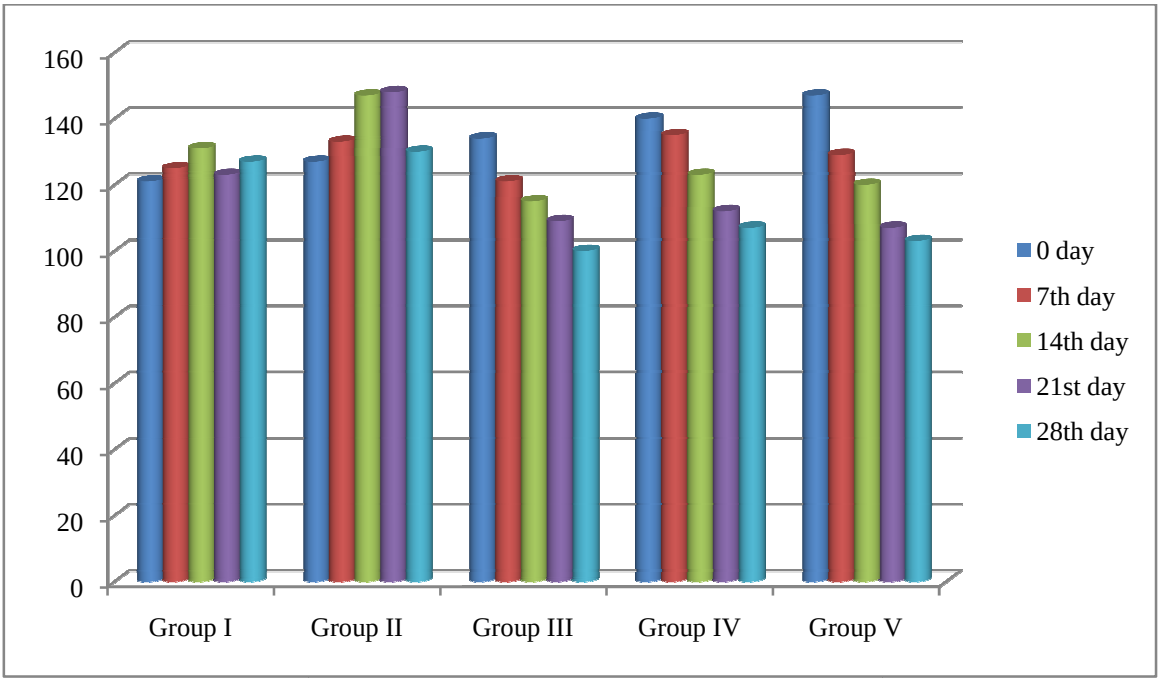


Fig 31. Graphical representation of changes in body weight (grams)

- ❖ Group I compared with Groups II, III, IV, V
- ❖ Group II compared with Groups III, IV, V

At the end of 28th day, ethanolic extracts produced a significant reduction of body weight of 67% at 200mg/kg and 76% at 400mg/kg compared to diabetic control. On the other hand, Glibenclamide produced a significant blood glucose reduction of 65% and the ethanol extracts brought about 54% at 200mg/kg and 57% at 400mg/kg.

Effect of ethanolic extract of *Delonix regia* (Boojer .Hook.). Raf., seeds on plasma lipid profile

Table 25. Plasma lipid profile

Treatment	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	Triglyceride (mg/dl)
Group I	94.66±5.8	48.00±4.17	77.40±4.46	26.50±2.58	81.16±6.24
Group II	215±4.84	27.50±3.30	169.00±5.51	58.83±4.57	190.50±9.62
Group III	90.50±12.78	51.83±3.97	85.83±4.44*	27.66±4.45*	79.17±4.44*
Group IV	101.83±4.62	48.00±4.51	123.83±5.87*	31.66±4.45*	96.16±5.45*
Group V	95.33±5.27	50.16±4.79	92.16±6.01*	29.66±4.45*	90.17±4.91*

One way ANOVA values are expressed as mean ±SD n = 6

*P < 0.05 compared to diabetic control

Note:

- Group I Normal control
- Group II Diabetic control
- Group III Standard drug (Glibenclamide 3mg/kg)
- Group IV Ethanolic extract of *Delonix regia* 200mg/kg
- Group V Ethanolic extract of *Delonix regia* 400mg/kg

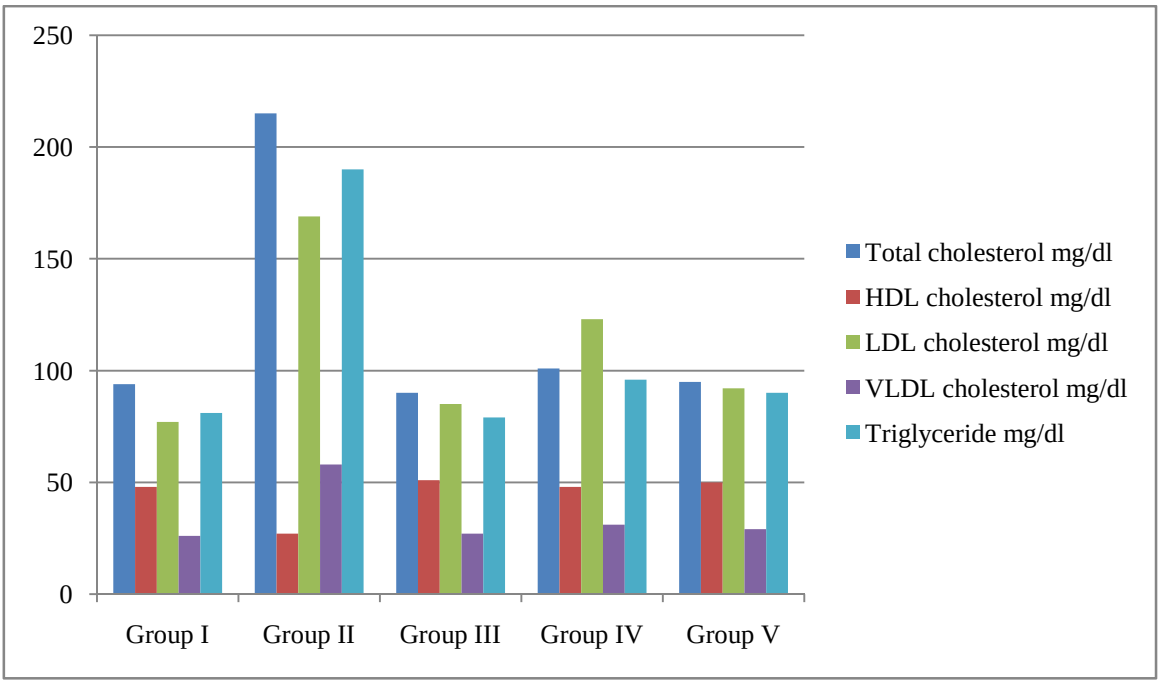


Fig 32. Graphical representation of lipid profile in study groups

- Group I compared with groups II, III, IV, V.
- Group II compared with groups III, IV, V.

HAEMATOLOGICAL PARAMETERS

Haematological parameters of ethanolic extract of *Delonix regia* (Boojer .Hook.). Raf., seeds

Table 26. Haematological parameters

Parameters	Group I	Group II	Group III	Group IV	Group V
RBC(cells/cumm)	6.77±0.24	4.94±0.24	6.52±0.08	5.91±0.01	6.21±0.12
WBC(cells/cumm)	7.53±0.12	11.05±0.51	7.62±0.14	7.89±0.25	7.58±0.51
Lymphocytes%	48.33±0.45	39.21±0.23	47±0.13	46.15±0.51	49.21±0.34
Monocytes%	4.17±0.14	5.81±0.61	4.27±0.09	4.87±0.21	4.34±0.15
Eosinophils%	2.06±0.74	1.64±0.21	1.89±0.04	1.74±0.84	2.04±0.46
Neutrophils%	45.42±0.19	54.14±0.53	47.05±0.14	46.01±0.17	45.04±0.08
Basophils%	0.00±0.00	1.33±0.57	0.12±0.04	0.25±0.51	0.10±0.34
PCV%	41.56±0.14	35.07±0.01	40.05±0.11	39.09±0.42	41.24±0.45
Hb(g/dl)	13.7±0.45	9.3±0.15	12.2±0.65	11.90±0.15	12.8±0.75

One way ANOVA values are expressed as mean ±SD n = 6

*P < 0.05 compared to diabetic control

Note:

- Group I Normal control
- Group II Diabetic control
- Group III Standard drug (Glibenclamide 3mg/kg)
- Group IV Ethanolic extract of *Delonix regia* 200mg/kg
- Group V Ethanolic extract of *Delonix regia* 400mg/kg

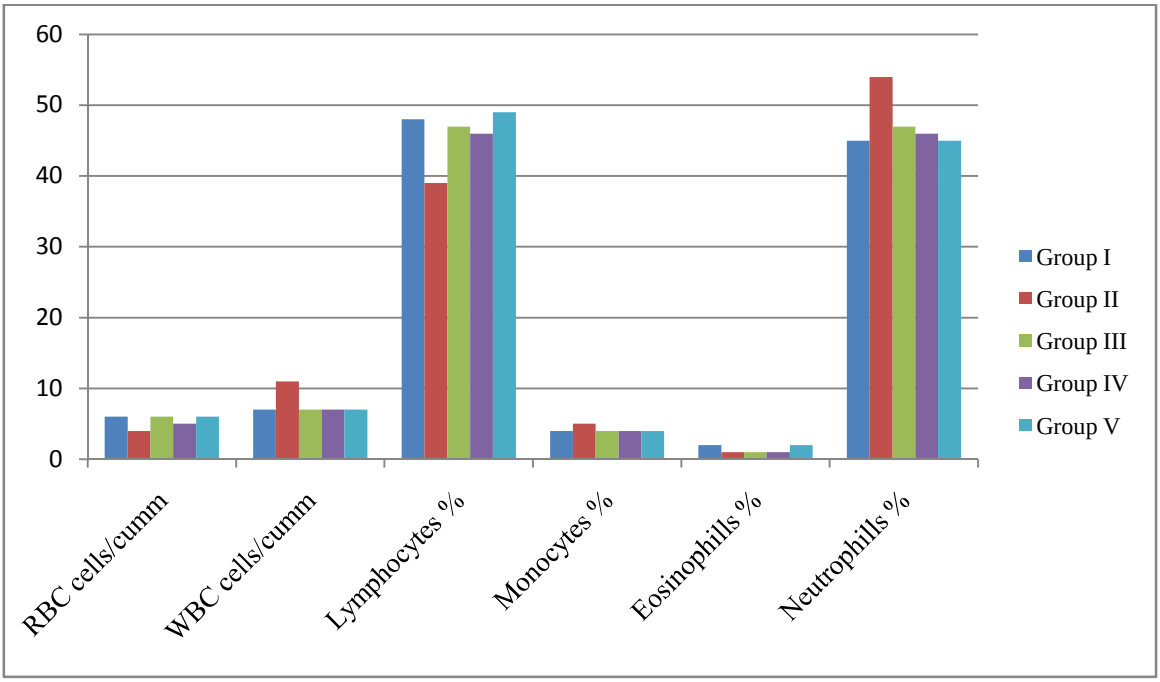


Fig 33. Graphical representation of Haematological parameters in study groups

- Group I compared with groups II, III, IV, V.
- Group II compared with groups III, IV, V.

HISTOPATHOLOGICAL EXAMINATION OF PANCREAS

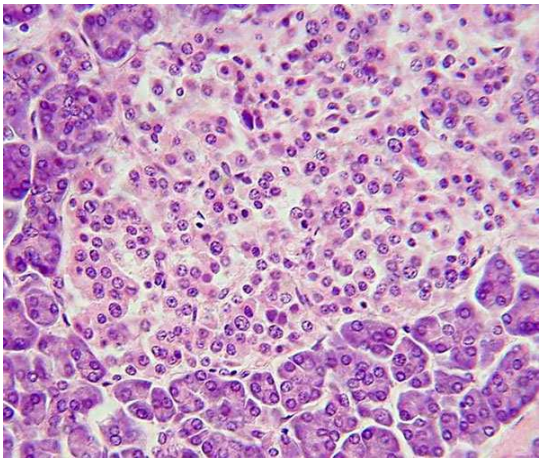


Fig 34. Group I

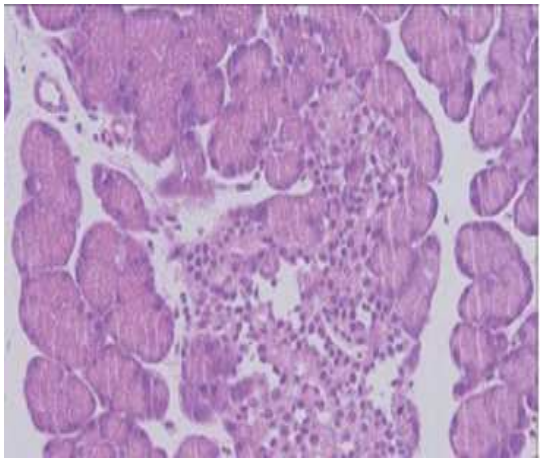


Fig 35. Group II

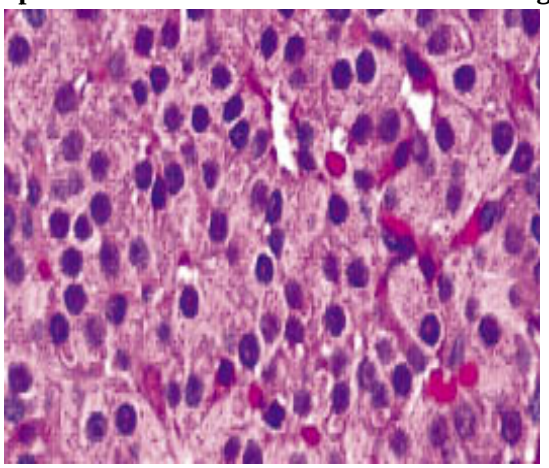


Fig 36. Group III

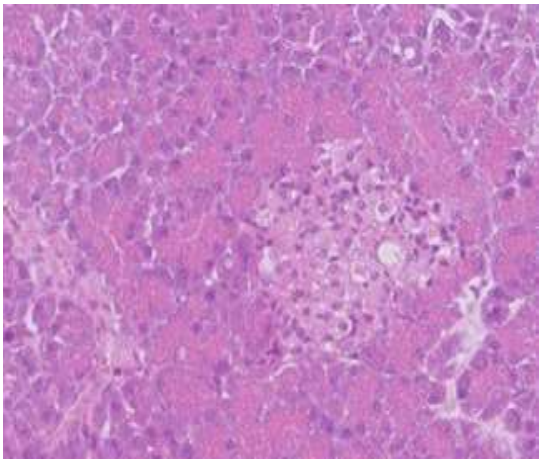


Fig 37. Group IV

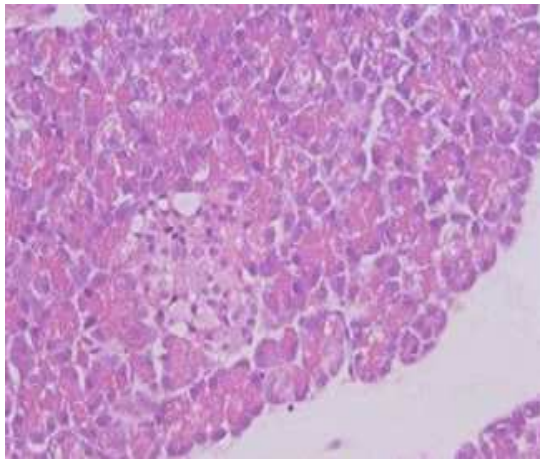


Fig 38. Group V

- Group I Normal control
- Group II Diabetic control
- Group III Standard drug (Glibenclamide 3mg/kg)
- Group IV Ethanolic extract of *Delonix regia* 200mg/kg
- Group V Ethanolic extract of *Delonix regia* 400mg/kg

ISOLATION AND CHARACTERIZAITION

COLUMN CHEROMATOGRAPHY

The elutes obtained from silica gel Column chromatography of Ethanolic extract with different fractions are tabulated in Table.

Table 27. Column chromatography of EEDR

S. No	Eluent	Solvent ratio	Fractions
1	Ethyl acetate	100%	1 – 3
2	Ethyl acetate: ethanol	90:10	4 – 6
3	Ethyl acetate: ethanol	80:20	7 – 9
4	Ethyl acetate: ethanol	70:30	10 – 12
5	Ethyl acetate: ethanol	60:40	13 – 15
6	Ethyl acetate: ethanol	50:50	16 – 18
7	Ethyl acetate: ethanol	40:60	19 – 21
8	Ethyl acetate: ethanol	30:70	22 – 24
9	Ethyl acetate: ethanol	20:80	25 – 27
10	Ethyl acetate: ethanol	10:90	28 – 30
11	Ethanol	100%	31 – 33

THIN LAYER CHROMATOGRAPHY

Solvent system

Hexane: Ethyl acetate: water in 3.5:2.5:0.5

Table 28. Thin layer chromatography of isolated fractions of EEDR

S. No	Fractions	Compounds	R_f value
1	1 - 3	-	-
2	4 - 6	-	-
3	7 - 9	-	-
4	10 – 12	-	-
5	13 – 15	-	-
6	16 – 18	-	-
7	19 – 21	-	-
8	22 – 24	-	-
9	25 – 27	-	-
10	28 – 30	Compound – 1 – 7	0.30 0.50 0.62 0.76 0.80 0.87 0.91
11	31 - 33	Compound – 1 – 7	0.30 0.50 0.62 0.76 0.80 0.87 0.91

The R_f value of the compound 1 – 7 was found to be 0.30, 0.50, 0.62, 0.76, 0.80, 0.87, 0.91

SPECTRAL ANALYSIS

The compound obtained with the ethanolic extract has been identified and spectral data were depicting in the figures

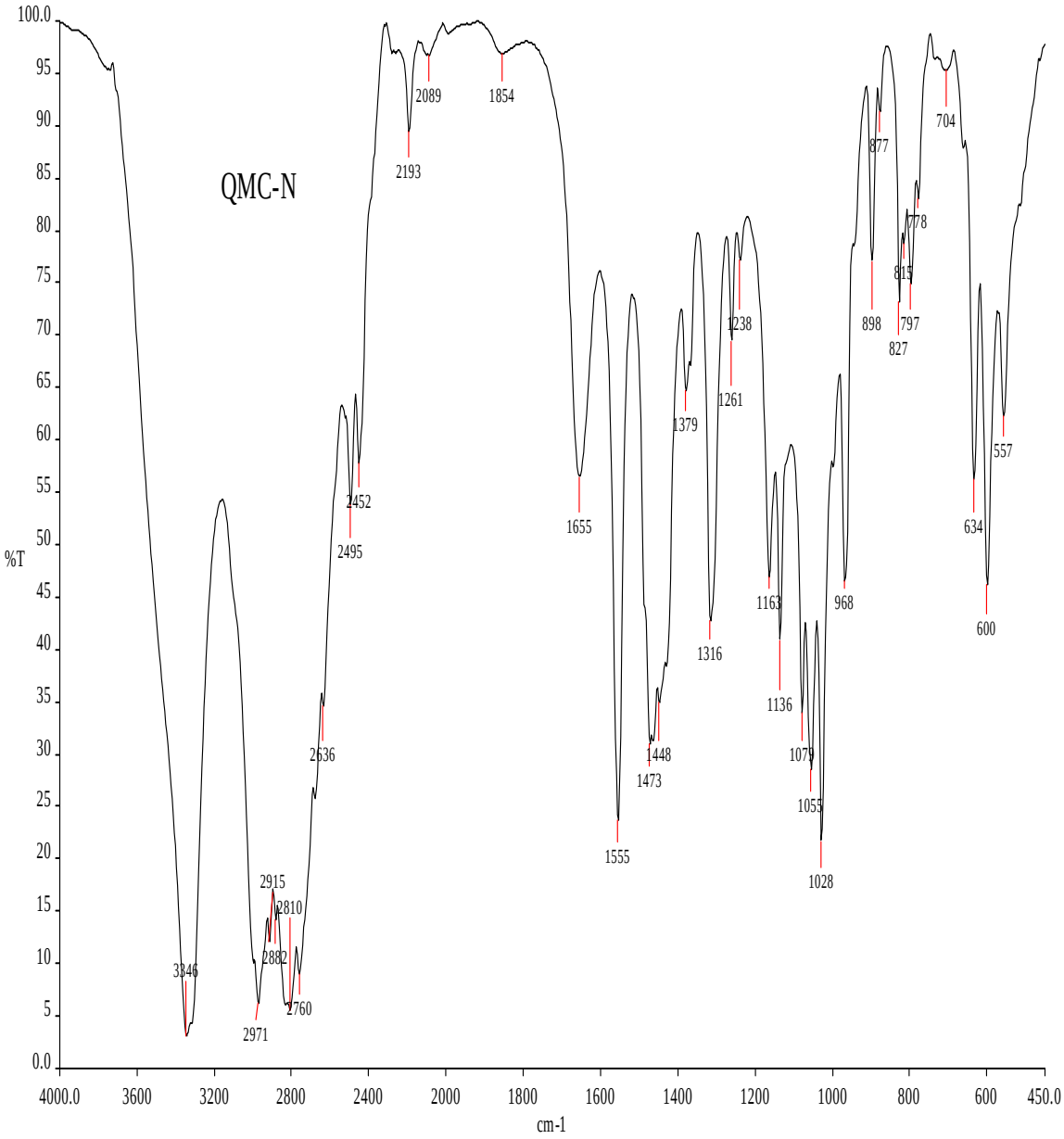


Fig 39. Compound: 1 – 7 IR spectra

Table 29. IR spectroscopy of Compound: 1 – 7 (IR υ cm^{-1}: Nujol mull)

Wave numbers cm^{-1}	Intensity	Type
1238	Moderate peak	Phenol
800 – 720	Moderate peak	*O* - substituted benzene
2915	Moderate peak	Benzene
1055	Sharp peak	C – O – C stretching
1600 – 1900	Moderate Peak	>C = O Carbonyl group
2810	Moderate peak	C – H stretching in CH_3
3346	Moderate peak	OH
1448	Moderate peak	Aromatic C = C stretching
1655	Sharp peak	Isolated C=C
1473	Moderate Peak	C – H stretching
1379	Moderate Peak	C – H methyl
2193	Sharp peak, weak band	C ≡ C
968– 704	Sharp peak	-CH = CH_2
600 – 800	Moderate peak	Halogen
2917	Sharp peak	Aliphatic CH
2495	Sharp peak	OH acid

Spectral analysis of compounds Compound: 1 – 7

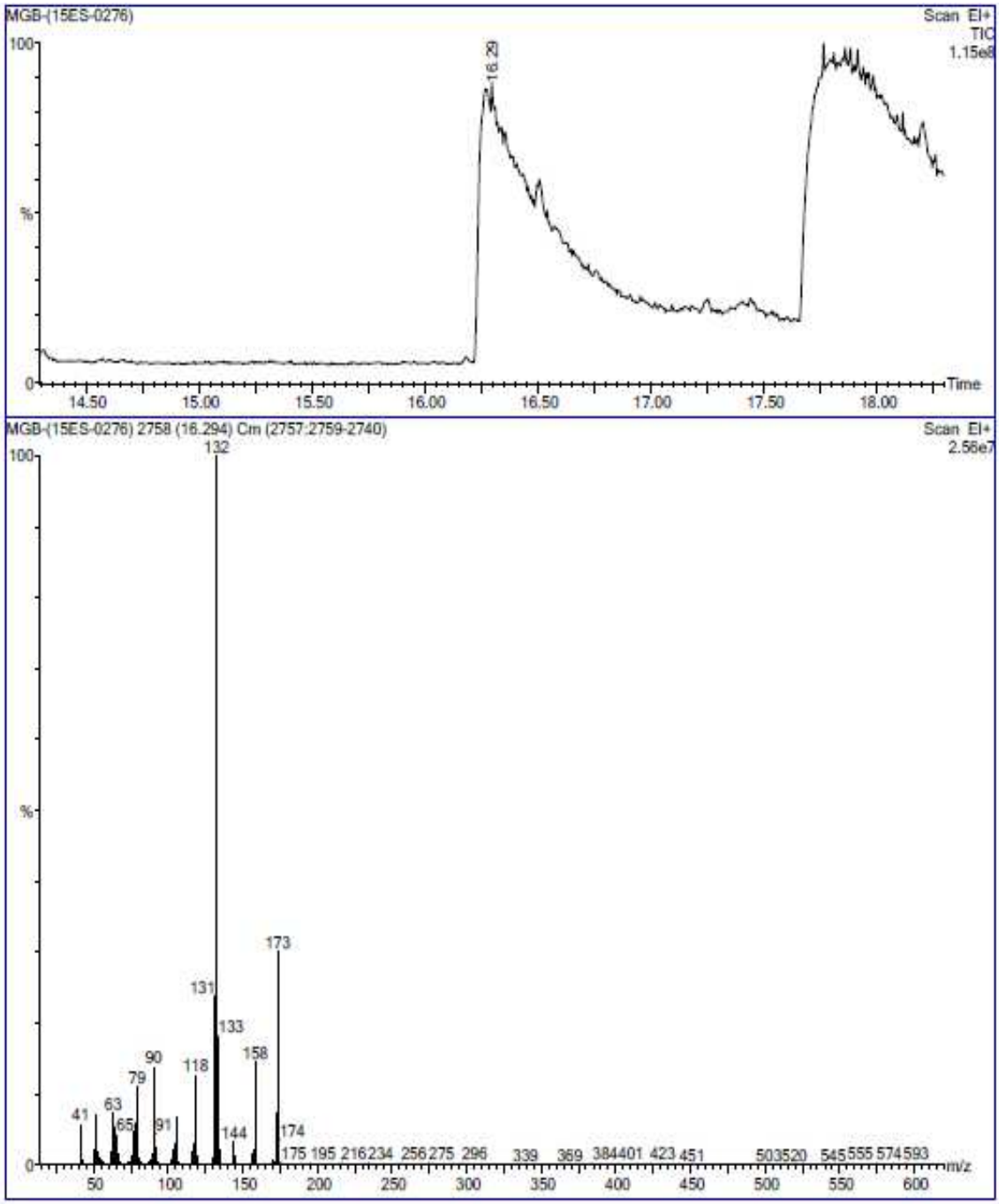


Fig 40. Compound-I GC-MS spectra

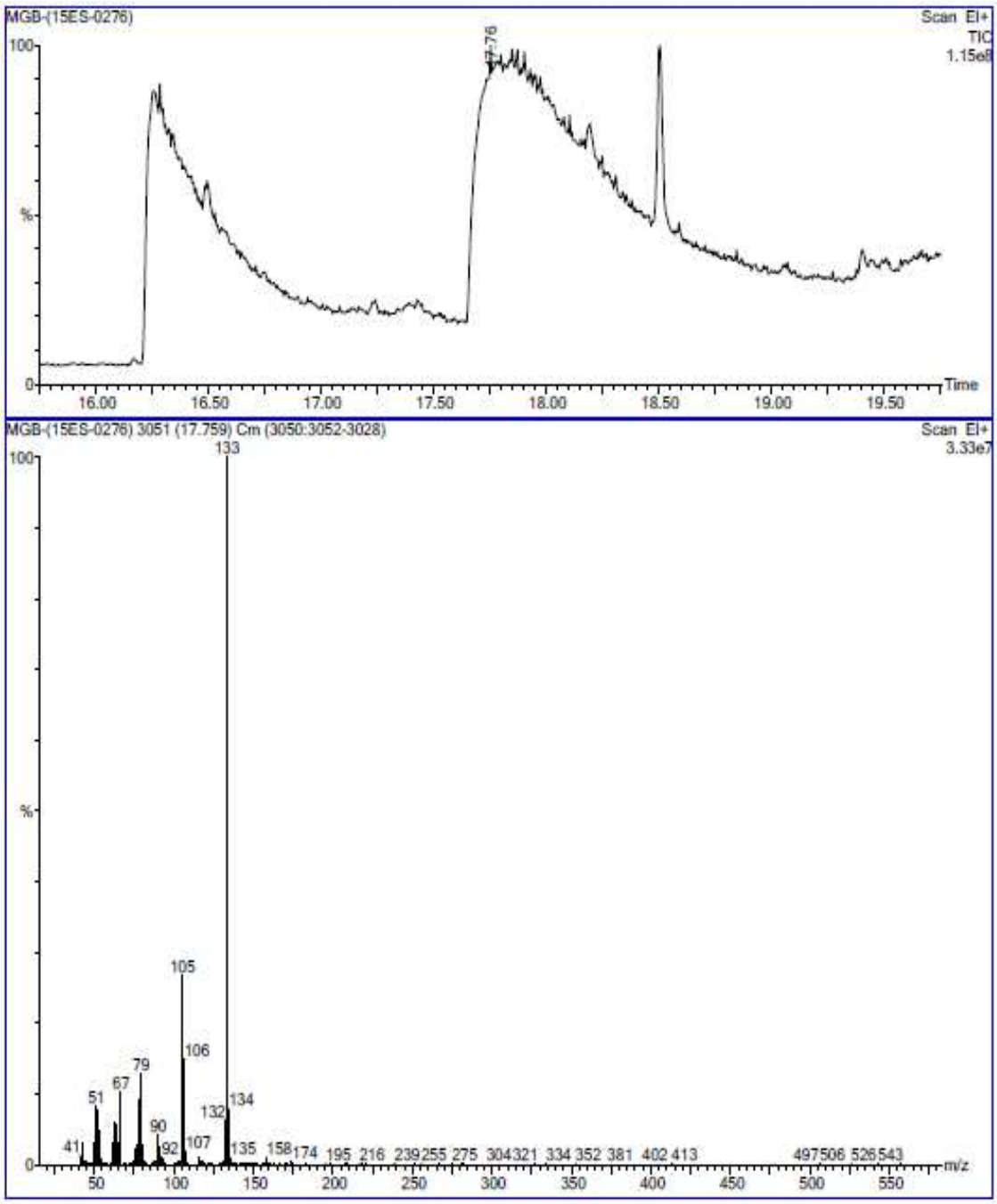


Fig 41. Compound-II GC-MS spectra

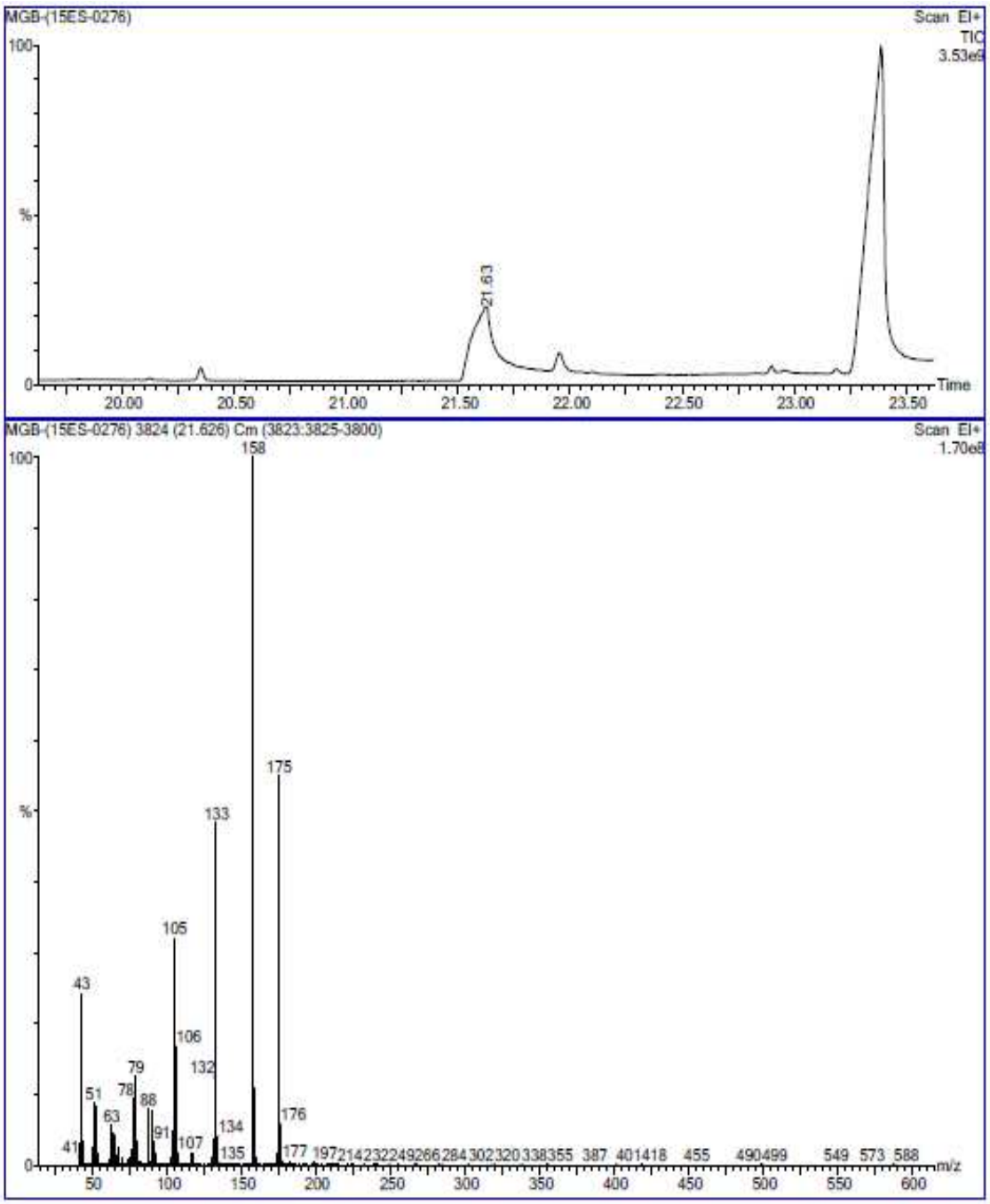


Fig 42. Compound-III GC-MS spectra

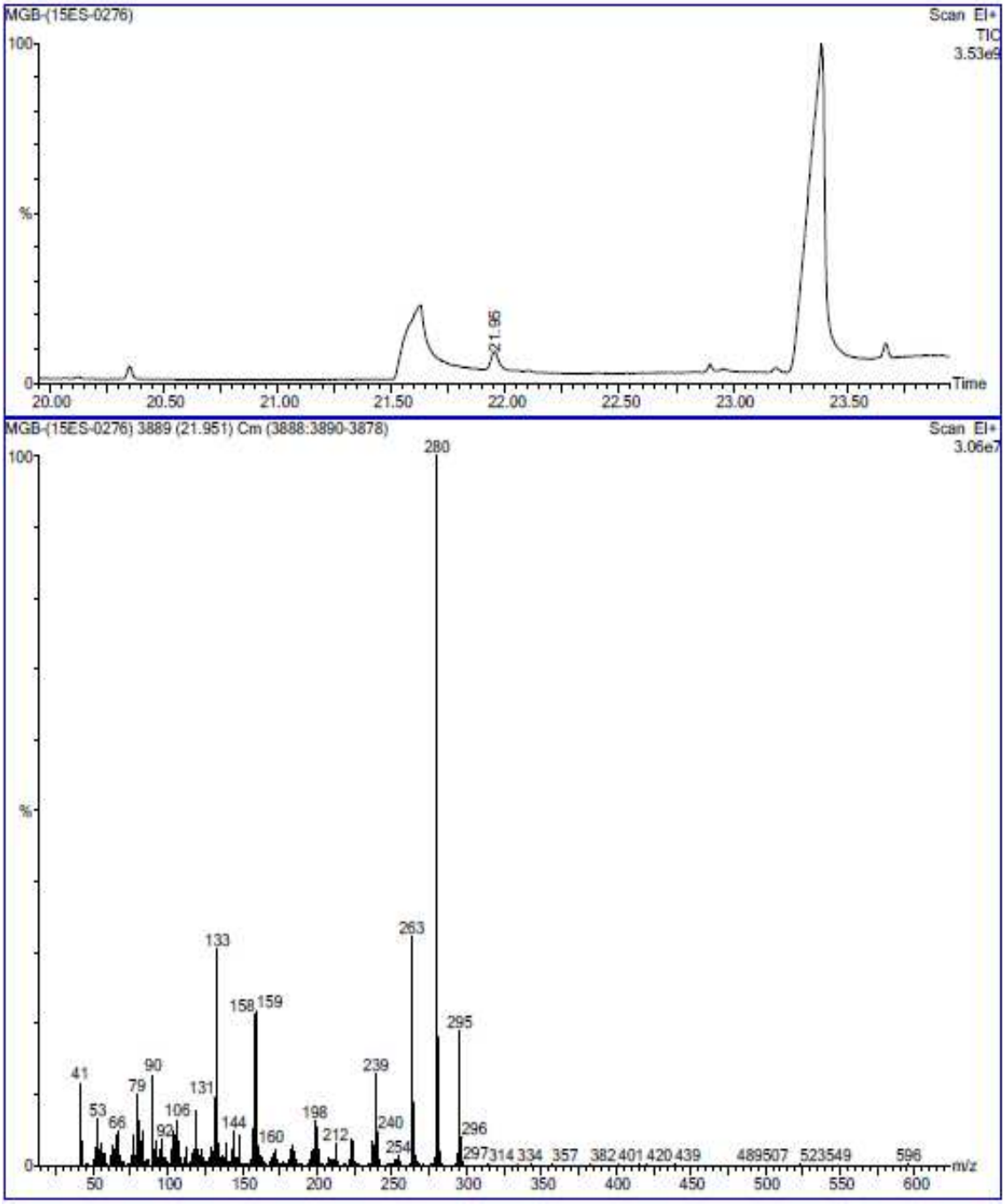


Fig 43. Compound-IV GC-MS spectra

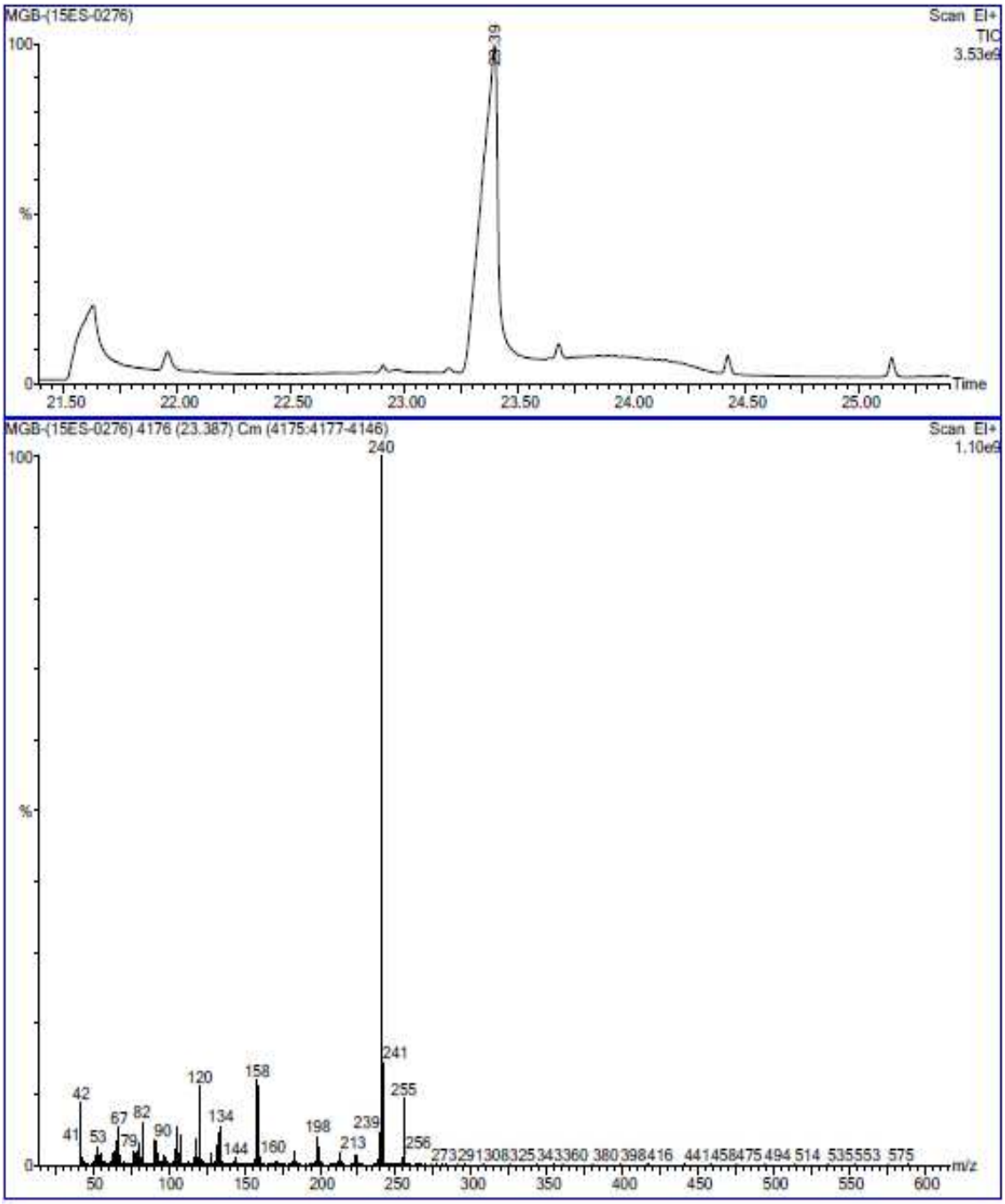


Fig 44. Compound-V GC-MS spectra

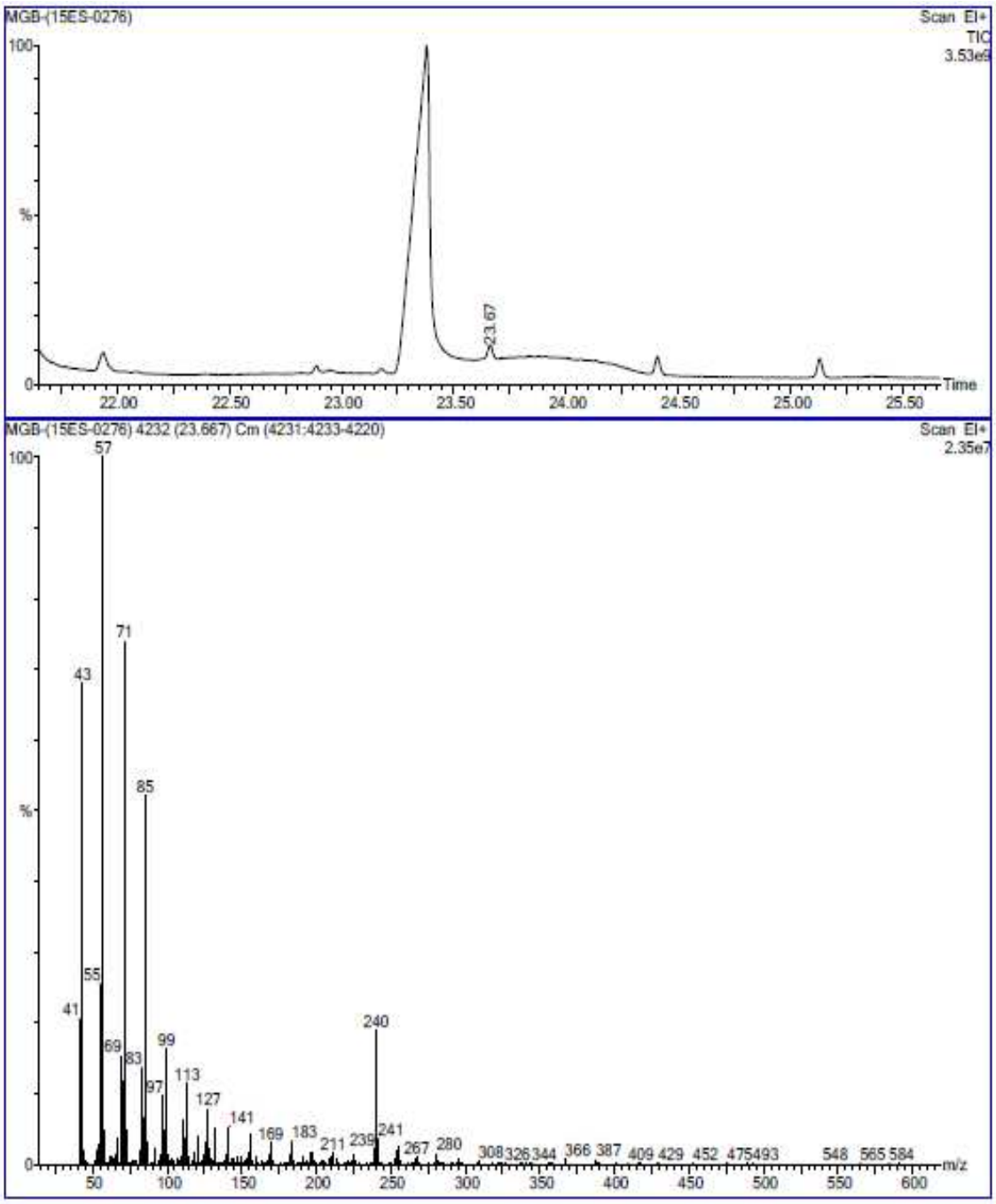


Fig 45. Compound-VI GC-MS spectra

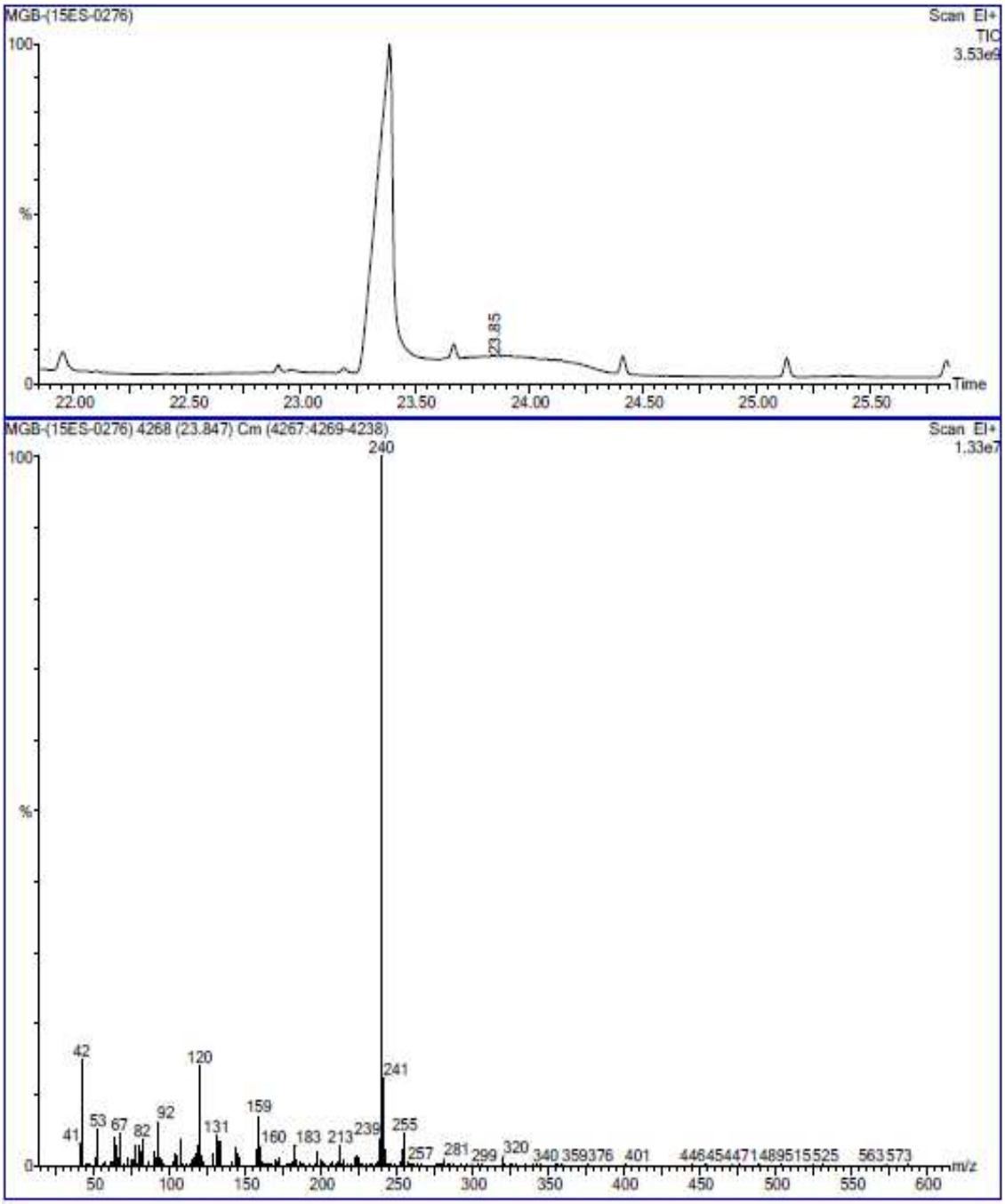


Fig 46. Compound-VII GC-MS spectra

GC-MS report showed the presence of seven compounds in the fraction 28-33. In future isolated fraction 28-33 will be further fractionated, characterized and studied for its activity.

SUMMARY AND CONCLUSION

9. SUMMARY AND CONCLUSION

The project entitled **"Pharmacognostical, Phytochemical and pharmacological studies on *Delonix regia* (Boojer .Hook.) Raf.," seeds, (Leguminosae)** has been achieved by the following results.

- The literature survey showed that only very scrappy information was available on this plant. With the scantly information on this plant and its folklore claim to be used for diabetic but has not been scientifically reported.
- In pharmacognostical studies authentication plays an important key. The *Delonix regia* (Boojer .Hook.) Raf., was collected from Nugambakkam, chennai and authenticated by M. Palanisamy, Scientist 'D'- In – Charge, Botanical Survey of India, Coimbatore.
- The pharmacognostical parameters were studied. Such as macroscopy, microscopy, powder microscopy. Histochemical studies and physicochemical constants to established.
- The qualitative and quantitative analysis was carried out to identify inorganic elements present in the plant. The qualitative and quantitative analysis of toxic heavy metals like cadmium, arsenic, lead and mercury were within the WHO limits and ensure the safety of the drug.
- The coarse powder was extracted by Hexane, Ethyl acetate, Ethanol by successive solvent extraction by hot percolation method and aqueous extract by cold maceration.
- The preliminary phytochemical screening of various extract and powder of the plant has revealed the presence of constituents in ethanolic extract like flavonoids, saponins, steroids and triterpenes.

- Qualitative chromatographic analysis – TLC for various extracts was carried out and HPTLC for ethanolic extract to conformed the phytoconstituents present.
- *In vitro* studies, DPPH radical scavenging assay and α – amylase inhibition assay performed for the selection of the bio active extract. Accordingly, the EEDR was possess antidiabetic activity. So selected for *in vivo* studies.
- The acute toxicity study was done. Based on this study as per OECD guidelines 425, the ethanolic extract was found to be very safe up to 2000mg/kg. Efficacy of the extract was screened by evaluation antidiabetic activity.
- Antidiabetic activity was assessed by streptozotocin induced non – insulin dependent diabetic mellitus. The parameters studied were oral glucose tolerance test (OGTT), hypolipidemic activity, blood glucose level, body weight changes, haematological parameters and histopathological studies.
- All parameter revealed the most effective EEDR was selected for column chromatographic separation in an attempt to isolate the compounds. The compound was isolated by column chromatography and then subjected to thin layer chromatography followed by spectral analysis. It was identified as **seven compounds.**
- From the above mentioned studies it can be concluded that the pharmacognostical reports generated will be useful for the proper identification of plant and also to differentiate it from closely related species and adulterants. With the support of *in vitro* studies and phytochemical screening the ethanolic extract were subjected to *in vivo* studies. The EEDR showed the significant antidiabetic activity.
- Further studies were focused on isolation and characterization of the phytoconstituents is responsible for antidiabetic activity.

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ANNEXURES

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
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टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2015/Tech. - 1696 दिनांक/Date: 7th August 2015

सेवा में / To

Ms. N. Shantha Sheela
II Year M. Pharm.
Department of Pharmacognocy
College of Pharmacy
Madras Medical College
Chennai – 600 003

महोदया/Madam,

The plant specimen sent by you for identification is identified as *Delonix regia* (Hook.) Raf. - LEGUMINOSAE. The identified specimen is returned herewith for preservation in their college/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

7.8.15

(डॉ. एम. पलनिसामी/Dr. M. Palanisamy)
वैज्ञानिक 'डी' प्रभारी/Scientist 'D'-In-Charge

वैज्ञानिक 'डी' / SCIENTIST 'D'
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003.

CERTIFICATE

This is to certify that Ms. **N. SHANTHA SHEELA**, M.Pharm II year, Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai – 600003 had submitted her protocol (Part B Application) 12/243/CPCSEA for the dissertation programme to the Animal Ethical Committee, Madras Medical College, Chennai – 600003.

TITLE: PHARMACOGNOSTICAL, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON SEEDS OF *Delonix regia* (Bojer. Exhook.) Raf.

The Animal Ethical Clearance Committee experts screened her proposal No: 12/243/CPCSEA and have given clearance in the meeting held on 10/08/2015 at Dean's Chamber in Madras Medical College, Chennai – 600003. Her study involves only Wistar rats.

Signature

4/8/2015

Dr. S.K. SEENIVELAN, B.V.Sc.,
Reg. No: 2175
SPECIAL VETERINARY OFFICER
ANIMAL EXPERIMENTAL LABORATORY
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003.

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE, CHENNAI-3.

Certificate

This is to certify that SHANTHA SHEELA. N (I-M.PHARM) PHARMACOGNOSY

College of Pharmacy, Madras Medical College has attended the guest lecture "Recent Trends in Herbal Research" on 13th June 2014 at the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai-600003.

Dr. N. Jayshree
Professor & Head,
Department of Pharmacognosy,
College of Pharmacy

Dr. A. Jerad Suresh
Principal
College of Pharmacy

MOTHER THERESA POST GRADUATE AND RESEARCH INSTITUTE OF HEALTH SCIENCES

(A Government of Puducherry Institution)

Indira Nagar, Gorimedu, Puducherry-605 006.

Recent Trends in Industrial Pharmacognosy

RTIP'15

Certificate of Participation

This is to certify that

Dr./Mr./Ms. SHANTHA SHEELA . N

has participated in the AICTE sponsored 4th National Conference on

"Recent Trends in Industrial Pharmacognosy - 2015"

held on 13th March 2015, at MTPG & RIHS, Puducherry.

V. Gopal

Prof. **DR. V. GOPAL**
Registrar (Academic) - MTPG & RIHS
Convenor - RTIP'15

Dr. R. Murali
Dean - MTPG & RIHS
Chief Patron- RTIP'15

Certificate No : RTIP15|MMC|COP|PG|232

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE
CHENNAI-3.

CERTIFICATE

This is to certify that Dr/Mr/Mrs/Miss Shantha Sheela . N *of*

Department of Pharmacognosy, College of Pharmacy, MMC *has participated in the*

The Tamil Nadu Dr. M.G.R. Medical University Sponsored "Guest Lecture Series - 2014" conducted on

26th & 27th September, 2014 at the College of Pharmacy, Madras Medical College, Chennai - 600003.

Dr. N. Jayshree
Co-Ordinator,
College of Pharmacy, MMC.

Dr. A. Jerad Suresh
Chair Person
Principal, College of Pharmacy, MMC.

66th Indian Pharmaceutical Congress Hyderabad

66th INDIAN PHARMACEUTICAL CONGRESS

23rd TO 25th, JANUARY 2015, HITEX, HYDERABAD.

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~~Dr.~~/ ~~Prof.~~/ ~~Mr.~~/Ms. SHANTHA SHEELA . N

HAS PARTICIPATED AS DELEGATE

IN 66th INDIAN PHARMACEUTICAL CONGRESS

HELD AT HITEX, HYDERABAD FROM 23rd TO 25th, JANUARY 2015.

RAVI UDAY BHASKAR	K. RAJA BHANU	P. VENKATESHWARLU
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This is to certify that

SHANTHA SHEELA. N

has participated as a Delegate
in the 67th IPC held at JSS University, Mysuru
19 - 21 December, 2015.

Dr. B. Suresh	Dr. B. Manjunatha	Dr. (Lt Col) R. Vijaysimha
Chairman, LOC	Organising Secretary	Chairman, Registration
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